

Abstract Book



SETAC Europe 36th Annual Meeting

17–21 May 2026 | Maastricht, The Netherlands

Embrace the Outlier: In Science, Regulations and Networks

Abstract Book

SETAC Europe 36th Annual Meeting

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This book compiles the abstracts from the 36th annual meeting of the Society of Environmental Toxicology and Chemistry – Europe (SETAC Europe), conducted from 17–21 May 2026 in Maastricht, the Netherlands.

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 high-lighted 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 Advancements in Data-Driven Ecotoxicology: Integrating Data and Innovative Methods

1.01.T-01 AIVIVE: Quantitative Comparison of Uncertainty in Hazard Characterization Using Traditional Animal Studies Versus New Approach Methodologies (NAMs) Leveraging AI-Driven Data Extraction

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Traditional chemical hazard assessment relies largely on in vivo studies summarised into point estimates such as NOAELs, EC values and benchmark doses (BMDs), which obscure underlying variability, uncertainty and mechanistic context. In contrast, New Approach Methodologies (NAMs), including high-throughput in vitro assays, omics platforms and computational tools, offer pathway level insight but remain difficult to integrate into regulatory frameworks due to heterogeneous data structures and uncertain comparability with traditional endpoints.

To address this gap, we propose an AI-enabled framework, using locally trained LLMs based on BioMistral, explainable AI (XAI) and aligned pipelines, to extract, harmonise and compare toxicological information from in vivo studies, NAM datasets and established resources such as ToxCast/Tox21 and AOP-Wiki. This supports large-scale cross-modality comparison of dose-response patterns and mechanistic concordance across chemical groups, aligning with current efforts to advance data-centric, non-animal toxicology.

A multilayer workflow retrieves and standardises traditional and NAMs derived data. Bioactivity datasets from CompTox follow ToxCast/Tox21 and PARC ontologies. In parallel, advanced NLP/LLM tools extract full dose-response information and metadata from scientific literature and regulatory dossiers, enabling reconstruction of experimental variability. Curated data are integrated into a semantic Knowledge Graph following FAIR principles.

The AI-driven extraction system successfully recovers study descriptors, dose-response datapoints and mechanistic annotations across heterogeneous formats, enabling harmonised datasets suitable for comparative modelling. Integration with ToxCast/Tox21 profiles and AOP-linked evidence yields mechanistic fingerprints consistent with established toxicodynamic frameworks. The harmonised dataset enables preliminary uncertainty evaluation and mechanistic clustering to explore whether chemicals sharing NAMs-derived pathway activation profiles exhibit coherent biological effects.

This work establishes a mechanistically grounded, uncertainty aware AI framework that enhances comparability between traditional and NAMs based evidence, supporting broader regulatory integration of NAMs and contributing to reduced reliance on animal testing in next-generation risk assessment.

1.01.T-02 Bio-QSARs: Enhancing Ecotoxicity Predictions by Exploiting Chemical and Biological Information

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Practical, legal, and ethical considerations necessitate the development of methods to replace animal experiments, commonly known as new approach methodologies (NAMs). Among these, machine learning (ML)-based quantitative structure-activity relationship (QSAR) models are a key component. Recently, we introduced the Bio-QSAR concept for multispecies aquatic toxicity regression tasks, enabling cross-chemical and cross-species predictions by integrating both chemical and biological descriptors. Here, we present extensions to the first-generation Bio-QSAR models that markedly expand the scope, data foundation, and performance of the initial approach.

First, training datasets were enlarged by approximately 20-fold, extending from pesticides to the broader chemical landscape and from freshwater organisms to aquatic species in general, while allowing for flexible test durations. Model training employed Gaussian Process Boosting, an algorithm combining tree boosting with mixed-effects modeling, to handle both fixed and random effects. We tested training Bio-QSARs using different

sources of biological information besides Dynamic Energy Budget (DEB) parameters by including, among others, taxonomic distances. The models achieved unprecedented predictive power, with R^2 values on independent test sets of up to 0.922 for fish and 0.905 for invertebrates. Applying SHapley Additive exPlanations (SHAP) in combination with a multicollinearity correction rendered the models fully explainable. Novel applicability domain (AD) approaches based on principal component analysis and feature importance improved predictive power and robustness by reducing outlier frequency. Next, we incorporated curated in-house toxicity data (~1,500 data points) into fish Bio-QSAR training, which further expanded the feature space, yielding higher predictive performance (AD-filtered $R^2 = 0.934$), and markedly fewer extreme outliers (0.4%). Our enhanced Bio-QSARs demonstrate flexible cross-chemical and cross-species prediction capabilities that meet OECD criteria for regulatory application. Current work focuses on extending the Bio-QSAR framework to algae and terrestrial plant species, paving the way toward a unified, multispecies modeling platform. These explainable, regulation-ready models hence hold promise to benefit a range of applications in environmental risk assessment as well as research and development of chemicals.

1.01.T-03 Data-Driven Mechanistic Forecasting of Species-Specific Chemical Sensitivity Using Docking-Derived Molecular Features

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Understanding cross-species susceptibility to chemicals remains a key challenge in environmental risk assessment (ERA), which still relies on a few model species and assumes others respond similarly. Conventional toxicity testing is slow and costly, limiting biodiversity coverage. To move from assumption to mechanism, we developed a framework that starts at the molecular initiating event (MIE) within an Adverse Outcome Pathway (AOP) and extends to organism and population effects. As proof of concept, we analysed chlorpyrifos and chlorpyrifos-oxon across non-model taxa by comparing their interactions with acetylcholinesterase (AChE) orthologs, linking variations in binding dynamics to susceptibility predictions and enabling more mechanistic ERA.

Molecular docking revealed consistently stronger target engagement for chlorpyrifos-oxon compared to chlorpyrifos, with protein-ligand interaction fingerprints aligning with empirical sensitivity rankings. When converted into calibrated susceptibility scores and compared with species sensitivity distributions, the framework identified known sensitive lineages such as cladocerans and amphipods, while highlighting plausible outliers among fish where toxicokinetics and barrier properties likely attenuate target-level effects. This defines a mechanistic link from molecular trigger to risk-relevant outcomes, distinguishes cases where strong target binding does not result in high sensitivity, and minimises false-negative classification of vulnerable species.

We then scale this logic to a broader endocrine axis. Using curated ecdysone receptor (EcR) orthologs from >1,200 arthropods, we simulate binding of synthetic diacylhydrazine (DAH) agonists and embed these signals in an EcR activation pathway. The evidence resolves hotspot clades, ranks DAHs above steroidal agonists in predicted affinity, and nominates early-warning biomarkers tied to molting transcriptional programs. The output is a regulatory-scale, traceable species–compound prioritisation to guide testing, monitoring, and mitigation. Limitations remain, coarse docking physics, uneven omics coverage, incomplete toxicokinetics; but the framework is modular and ready to absorb better receptor dynamics, metabolism, and additional off-target receptors. Combined, this framework provides mechanistically anchored evidence for New Approach Methodologies (NAMs) and Next-Generation Risk Assessment (NGRA), linking molecular triggers to ecological effects.

1.01.T-04 Ruleless Reaction Prediction with Transformers utilising Enzyme Context

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Enzyme-catalysed reactions are common in many areas, including pharmaceutical metabolism and agricultural chemical biodegradation. Analysing and predicting how these reactions occur is increasingly important for identifying toxic by-products and achieving regulatory approval. Incorporating enzyme information into these predictions has been shown to improve prediction capabilities. However, existing methods require knowledge of the enzyme to perform prediction, and in many situations, especially biodegradation, the complexities of the reaction environment mean the exact enzymes are not known. In this presentation, we propose a hierarchical multi-label classifier and associated evaluation framework to predict the enzyme commission number a

chemical compound reacts with. Our method achieves a hierarchical F1-score of up to 93.2%, outperforming existing methods. Additionally, we present an ablation study on the effects of including enzyme information for product prediction.

1.01.P Advancements in Data-Driven Ecotoxicology: Integrating Data and Innovative Methods

1.01.P-Mo001 CRANE-DB: A Curated Dataset and Variability Analysis for Avian Acute Toxicity.

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Bird populations across Europe are declining despite their essential role in ecosystem services, with pesticide and fertilizer use identified as major drivers. Avian acute toxicity testing therefore remains central to pesticide risk assessment for non-target organisms. Although acute dietary testing has been waived and replaced with acute oral testing, this method remains low-throughput. At the same time, interest in New Approach Methods (NAMs) is increasing to reduce animal use, yet their implementation in avian toxicity studies is limited. A key barrier is concern about their relevance to in vivo data, making high-quality curated in vivo datasets crucial for NAMs development.

We curated avian acute oral toxicity data from the Toxicity Values Database (ToxValDB, v9.5), the Pesticide Properties Database (PPDB), and the U.S. Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP). Records were restricted to oral LD50 values with 14-day observation according to Organization for Economic Co-operation and Development Test Guideline 223. ToxValDB entries were manually checked against original sources to extract missing substance purity information, new LD50 values (lethal dose causing 50% mortality) and correct reporting errors. PPDB was limited to high-quality regulatory entries, and substance purity information was harmonized. Duplicates were removed within and across databases based on identical CASRN (CAS Register Number), species, LD50 value, purity, and qualifier.

Variability in LD50 values for compounds with multiple datapoints was assessed using median absolute deviation (MAD) and LD50 max/min ratios (>3 and >10). The effects of purity and the use of more than one species on variability were evaluated using Generalized Linear Mixed Models.

We retrieved 506, 466, and 889 records from ToxVal, PPDB, and OPP, respectively. After curation and de-duplication, our dataset contained 1671 entries covering 811 CASRN, which we named CRANE-DB. Majority of our records (55.2%) were from Northern Bobwhite, followed by 28.2% Mallard Duck. Nearly half of our entries (48.5%) have 2 or more LD50 within one CASRN. Furthermore, we examined LD50 variability within CASRN and found that low substance purity and cross-species testing significantly increase variability ($p < 0.05$). Purity effects were more pronounced within same species. These curation and analysis highlight CRANE-DB value as a more robust benchmark for developing NAMs in avian toxicity.

Disclaimer/Disclosure:

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1.01.P-Mo002 Threshold of Toxicological Concern (TTC)- refining ecological TTC using Enhance Database of Fragrance Chemicals.

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The Threshold for Toxicology Concern (TTC) is a well established and accepted concept in human health safety. Its purpose is to rapidly screen and assess data poor materials and mixtures by establishing an exposure concentration below which negligible risk is expected. The ecological threshold of toxicologic concern (ecoTTC) was created back in 2015 (Belanger et al. 2015) and proposed as a means to summarize larger amounts of ecotoxicity data with the purpose of Predictive No Effect Concentration (PNEC) extrapolation. Leveraging database with computational tools (Ecotoxicological Information System, EnviroTox; Connors et al. 2019) ecoTTC allows the prediction of 5th percentile PNEC for ecosystems using ecotoxicity data extrapolated to individual PNEC for individual compounds. Since TTC has been used by Research Institute for Fragrance Materials (RIFM) to screen fragrance materials in human health assessments, an effort began to develop an ecoTTC for fragrance materials. A review of available ecotoxicological data in the RIFM database as well as

additional literature including Europea Chemicals Agency (ECHA) registration database was used to derive ecological Thresholds of Toxicologic Concern (ecoTTC) and Chemical Toxicity Distributions (CTD). For the purpose of this project the following ecoTTC assessments and comparisons were made: material alone versus supplementation by EnviroTox; groupings based on chemical class, Verhaar Mode of Action (MOA) and MOATOX MOA classification, groupings based on various bands of log Kow, etc. In addition US-like and European-like ecoTTC assessments were evaluated. A total of 1094 ecotoxicity studies of all types were summarized covering 424 fragrance materials and results from analysis are going to be reported during the presentation.

1.01.P-Mo003 Integrating Ecotoxicity Evidence: A Meta-analysis of Pharmaceutical and Personal Care Product Effects on Freshwater Fauna

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Pharmaceuticals and personal care products (PPCPs) are increasingly detected in freshwater ecosystems due to their widespread use and incomplete removal during wastewater treatment. These chemicals include psychoactive substances, antibiotics, anti-inflammatory drugs, hormones, chemotherapeutic drugs, fragrances, preservatives, UV filters, plasticizers etc. Numerous studies report effects on survival, growth and reproduction in non-target freshwater organisms. However, the synthesis of such studies that would summarise the overall evidence of these effects has not yet been conducted. We thus conducted a systematic review and meta-analysis to integrate ecotoxicity evidence across freshwater taxa. Our main aims were to quantify the overall effects of environmentally relevant PPCP exposures on fitness of freshwater fauna, and to test several hypotheses on the variation in the magnitude of the effects. Fitness was defined as any direct or indirect endpoint associated with the survival or reproductive output of an organism. We identified 92 relevant peer-reviewed experimental studies and openly available datasets on 27 species. We calculated two effect sizes, log response ratios (lnRR) to estimate changes in mean trait values and log variance ratios (lnVR) to evaluate shifts in trait variability. Meta-regression models were used to assess moderators such as developmental stage, sex, exposure duration and environmental conditions on the magnitude of the effects. Additionally, we identified taxonomic and geographic gaps to provide directions for future studies. Finally, we assessed whether the included studies influenced policy or regulatory attention by exploring their alternative impact metrics. Our work provides a comprehensive quantitative synthesis of PPCP effects on freshwater animals. By integrating heterogeneous ecotoxicity data, our findings support improved prioritisation of PPCPs and strengthen the evidence base for environmental risk assessment and regulatory decision-making.

Disclaimer/Disclosure:

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1.01.P-Mo004 Curation of REACH Acute and Chronic Fish Toxicity Data and Assessment of Models Predictive Performance

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Under REACH, both acute and chronic fish toxicity data are required for the hazard and risk assessment of substances manufactured or imported at 10 ton/y or more, yet experimental testing is resource-intensive and often complicated by substance properties (e.g. low solubility). Hence, it is important to maximize the use of already generated data, e.g. to validate alternative in vitro and in silico methods, establish the relative species sensitivity and assess the role of fish acute and chronic studies in hazard assessment.

We constructed high-quality reference datasets fish toxicity by extracting and rigorously curating experimental studies from REACH registration dossiers, with AI-assisted algorithmic curation applying strict criteria for study reliability, guideline adherence, and molecular structure assignment. Acute and chronic effect levels were standardised and categorised into scenarios reflecting test design and data quality. The dataset comprises approximately 700 substances with LC₅₀ values derived from high-quality short-term fish toxicity, fish embryo, or fish cell line assays. These values were obtained through concentration - response modelling based on analytical verification of concentrations. For long-term toxicity, the dataset includes NOEC/EC₁₀ values for

around 250 substances, generated using tests such as the fish early-life stage toxicity test, juvenile growth test, life cycle toxicity test, short-term embryo and sac-fry stage test, or fish sexual development test, all conducted under equally rigorous reliability criteria.

As a first use of the reference datasets, we assessed the accuracy and applicability domain coverage of predictive models, including Vega, ECOSAR, iSafeRat, T.E.S.T., ISIDA and KATE. Models were benchmarked for their ability to predict effect concentrations and regulatory classification thresholds both globally and within chemical subspaces using spectral clustering. Several models demonstrated reasonable predictive accuracy for in-domain chemicals. For acute toxicity 80-90% of the LC50s were predicted within 1 log unit. For chronic the performance was lower and with a much smaller applicability domain coverage. The analysis shows promise for using modelling in aquatic toxicity hazard assessment, but also highlights limitations due limited applicability domain, algorithm variance and bias and confounding factors, such as solubility limitations, that are not always and sufficiently accounted for by current models.

1.01.P-Mo005 Time to Shine: Illuminating How Ecotoxicology Laboratory Lighting Modulates the Circadian Metabolome

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Organisms rely on circadian clocks to optimise their metabolism, ensuring energy availability matches metabolic need and preventing contradictory metabolic processes from operating simultaneously (e.g. synthesis and degradation). The circadian clock regulates diverse metabolic processes like sleep and wakefulness, appetite and gut motility, and a suite of behavioural and physiological phenomena. In animals whose habitats are exposed to sunlight, light is generally the dominant signal for setting the circadian clock. Human studies show that limited access to the right quality and quantity of light is a major contributor to poor metabolic health. Thus far, there has been very little investigation into how indoor lighting affects the circadian metabolism of laboratory animals, despite often similar light quality to human-inhabited indoor spaces. In this study, we exposed Eastern mosquitofish (*Gambusia holbrooki*) to the typical fluorescent lights found in an environmental toxicology laboratory as well as light-emitting diodes (LEDs) with a spectral power distribution mirroring sunlight, and programmed with a simulation of sunrise and sunset. We used nuclear magnetic resonance and liquid chromatography-mass spectrometry metabolomics and lipidomics to investigate the effects of these lighting schemata on the fish's metabolic health. We ran a parallel field study to investigate which laboratory lighting schemata led to the most natural circadian metabolome. Using these data, we elucidated ways to improve laboratory design for animal health, to maintain circadian entrainment that reflects natural metabolic oscillations of wild fish, and to mitigate inadequate light quality as a confounding variable with sensitive multiparameter omics studies.

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1.01.P-Mo006 A multi-task neural network approach for predicting toxicokinetics in freshwater crustaceans

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This study develops a multi-task neural network-assisted toxicokinetic (TK) model to predict time-varying internal concentrations of organic compounds in freshwater crustaceans (water fleas and amphipods), particularly when compound-specific TK information is limited. The work includes: (i) establishing a comprehensive database of TK data for pharmaceuticals, pesticides, PFAS, and other organic contaminants; (ii) developing machine learning (ML)-assisted quantitative structure-activity relationship (QSAR) methods to identify physicochemical, biological, and environmental descriptors and to predict uptake (ku) and elimination (ke) rate parameters; and (iii) integrating these predicted parameters into a mechanistic TK model to form an ML-assisted TK framework. To stabilize variance and reduce the influence of extreme values, TK parameters were transformed prior to modeling, resulting in robust predictive performance in the transformed space. Because transformed values only reflect the learning space of the model, original-scale TK parameters were reconstructed for downstream TK simulations. Although uptake rates span several orders of magnitude, which naturally leads to larger absolute errors, especially for highly bioaccumulative compounds, the model still produces reliable predictions for both TK parameters in the original scale. SHAP analysis showed that life stage,

exposure duration, and dissolved organic matter strongly influence both k_u and k_e , while certain chemical descriptors contributed more prominently to elimination, indicating slightly greater sensitivity to intrinsic molecular properties. Validation demonstrated that the ML-assisted TK model captures time-varying internal concentrations and key TK features for most compounds, including peak concentration, time to peak, and overall exposure (AUC), with only a few cases showing poor agreement. Overall, this approach integrates chemical knowledge, biological context, and experimental data to fill critical TK data gaps and provides a mechanistically meaningful foundation for downstream TK applications.

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1.01.P-Mo007 From Variability to Validation: A Bayesian Approach for Evaluating Alternative Fish Toxicity Tests

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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”. Implicit in this, is understanding the inherent variability of traditional acute fish toxicity (AFT) studies, which has been largely unknown, and widely taken for granted. In this work, we developed a Bayesian hierarchical linear model applied on a curated database of over 1,000 historical AFT results. We leveraged our bespoke Bayesian model to both gain inference on the primary sources of variability in AFT studies and to derive a robust and statistically principled global margin of error (MOE) on LC50 endpoints, providing a quantitative benchmark against NAM-derived toxicity values. When applied across chemicals and species, our framework demonstrated that FET endpoints generally fall within the expected range of AFT variability, supporting their use as scientifically credible alternatives. Together, these findings provide a transparent, data-driven foundation for assessing NAM reliability and set the stage for reproducibility-based performance criteria in future validation and regulatory decision-making.

1.01.P-Mo008 Application of Statistical Causal Discovery to Development of Adverse Outcome Pathway

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The adverse outcome pathway (AOP) framework has evolved as a promising tool for organizing mechanistic toxicology information, providing a structured representation of the biological key events (KEs) that link a molecular initiating event (MIE) to adverse outcomes (AO) at higher levels of biological organization (e.g., death, reproductive impairment). Most published AOPs are still developed by expert judgment, although several semi-automated techniques have recently been proposed. Consequently, KE selection and causal directions are often inferred manually, and the resulting networks can be incomplete and subjective. As the volume of mechanistic toxicology data grows and the number of chemicals increases, there is an urgent need for data-driven approaches that can propose new AOP structures and verify existing ones. Statistical causal-discovery (SCD) methods offer such a data-driven solution. These algorithms infer causal structure directly from data, by imposing tractable assumptions that restrict the search space of possible causal graphs. However, ecotoxicology data often has challenges for SCD applications, such as missing data and violation of SCD algorithm assumptions.

As a proof-of-concept application of SCD to AOP development, we applied a linear non-Gaussian acyclic model (LiNGAM), a representative SCD method, to three types of ecotoxicology datasets: (1) bivariate dose–response relationships, (2) bivariate response–response relationships, and (3) a multivariate dataset with a known causal structure. Missing data were addressed through multiple imputation followed by causal estimation using DirectLiNGAM, a direct method for estimating LiNGAM. DirectLiNGAM identified correct causal directions with high statistical reliabilities in three of four bivariate dose-response cases, even when assumptions such as linearity and non-Gaussianity were partially violated. In contrast, response–response cases did not yield a single dominant direction, likely due to the limited number of replicates. In the multivariate case, the inferred graphs closely resembled the expert-curated causal graph, achieving high recall (0.50–0.75), despite relatively

low precision (0.31–0.40). These results demonstrate the utility of SCD, combined with multiple approaches, in identifying relevant key events, revealing missing links, and refining existing AOPs and quantitative AOP (qAOP) networks under realistic ecotoxicological constraints.

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1.01.P-Mo009 Comprehensive Transcriptomic Response Index for Decoding Nanomaterial-Induced Pulmonary Responses

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Engineered nanomaterials (ENMs) are commonly used in various industrial fields. Such widespread application has raised numerous concerns about the ENMs' safety. Currently, toxicogenomic studies play a central role in the ENMs hazard evaluation. However, omics datasets remain highly dimensional, which complicates their analysis and predictive modelling. In this work, we aimed to address this problem by developing a new transcriptomic response index (TRI). TRI compresses complex transcriptomic space, including hundreds of differentially expressed genes (DEGs), into a single predictive variable, while preserving linear interpretability with respect to gene-level contributions. Thus, we propose a biologically meaningful method in computational nanotoxicology that significantly contributes to new approach methodologies (NAMs) by reducing the need for animal testing to perform mechanism-based predictions.

We utilized previously published in vivo exposure data for titanium dioxide nanoparticles (TiO₂-NPs) and multi-walled carbon nanotubes (MWCNTs). The resulting datasets included transcriptomic responses following mouse inhalation exposure to TiO₂-NPs (621 differentially expressed genes, DEGs) and MWCNTs (5167 DEGs), alongside ENMs' physicochemical properties. We used principal component analysis (PCA) to estimate TRI based on the compressed transcriptomic data. Then we applied ridge regression to link the TRI to the inhaled ENMs properties. The amount of DEGs covered by TRI was defined based on the machine learning (ML) model robustness. The resulting ML models successfully approximate and predict over 5000 DEGs for MWCNTs (99.9% of the entire DEGs space, $Q^2_{\text{test}}=0.78$) and ~272 DEGs (~44% of the DEGs space, $Q^2_{\text{test}}=0.79$) for TiO₂-NPs datasets. Across this space, TRI links the expression of DEGs from Saa, Ccl, Cxcl, and other immune-related gene families to the properties of inhaled ENMs. The estimation of this relationship significantly contributes to the understanding of the mode of action of the pulmonary pathologies induced by ENM inhalation.

In line with SETAC's overarching objectives, our study advances the adoption of data-driven and mechanistically informed methodologies for chemical safety assessment by demonstrating a robust NAM-aligned omics endpoint for ENM hazard evaluation that interprets complex transcriptomic data and enhances mechanistic understanding of ENM-induced effects.

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1.01.P-Mo010 A Review of Quantitative Structure–Activity Relationship Approaches to Predict Avian Acute Toxicity

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Acute avian toxicity is assessed in vivo for all pesticides to be registered. It requires at least 30 birds per chemical and species, following OECD TG (Organisation for Economic Co-operation and Development Technical Guidelines) 233 and USEPA (United States Environmental Protection Agency) 850.2100 guideline, respectively. To reduce animal suffering, replacing these studies with New Approach Methods (NAMs) is desirable. In silico tools, particularly quantitative structure activity relationship (QSAR) approaches, offer a high-throughput and low-cost alternative. Several QSAR models for avian acute toxicity exist, yet none has been adopted in regulatory practice. In this study, we reviewed existing QSAR models based on ecotoxicological criteria (scope of the study, bird species, adherence to OECD guidelines, observation duration), modelling aspects (feature engineering, data pre-processing, algorithms), and model performance (statistical evaluation, applicability domain, biological plausibility). For four models, we attempted to re-implement the code and (1)

reproduce the published data and (2) run the model on our own, larger set of in vivo data (over 800 chemicals). Of fifteen publications initially considered, five were excluded for not addressing acute toxicity. For the ten remaining publications, the main limitations were: (i) no disclosure of training datasets (chemical features and in vivo data), (ii) insufficient reporting of model details for re-implementation, (iii) small datasets (10–50 chemicals), and (iv) misleading performance metrics, with high accuracy but poor sensitivity, indicating a limited ability to detect toxic compounds. We successfully replicated the reported results for most studies when using the preprocessed data files provided by the authors. However, recreating the full workflow from scratch often failed due to missing data, reliance on paid software, or random variation in feature selection algorithms. Despite multiple attempts, no QSAR model for acute avian toxicity is yet suitable for broad regulatory use. Insufficient methodological detail remains a key barrier, which could be mitigated by adhering to established QSAR reporting guidelines (e.g., OECD Nos. 69 and 386). Expanding the dataset of available in vivo toxicity values would further support model development and help pave the way toward regulatory application.

1.01.P-Mo011 Decoding ER α -azole derivative interactions to evaluate environmental health risks through computational and experimental models

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The widespread use of azole fungicides in agriculture is essential for crop protection, yet it has raised significant environmental and public health concerns owing to their potential endocrine-disrupting properties. This study investigates the molecular-level interaction of selected azoles with the estrogen receptor alpha (ER α) to elucidate their capacity to interfere with the receptor's native function. Our integrated computational approach began with molecular docking of the azoles alongside the native agonist estradiol (E2) and the antagonist 4-hydroxytamoxifen (OHT). The binding affinities of the resulting complexes were quantified using MM/GBSA analysis. The top five azole candidates were further characterized through ADMET profiling, density functional theory (DFT) calculations, molecular dynamics simulations (MDS) and post-simulation MM/GBSA calculations to visualize the dynamics of complex stability and interaction patterns. Furthermore, experimental validation using a BRET-based assay demonstrated that these azoles significantly suppressed E2-induced dimerization of cytosolic ER α . As expected, OHT exhibited a potent inducing effect. Collectively, these results underscore the need for focused strategies to mitigate the reproductive risks posed by azoles and to support environmental health.

1.01.P-Mo012 Intraspecies Correlation Estimation (ICE) Models for Acute-to-Chronic Toxicity Prediction of Insecticides to Daphnids, Chironomids, and Honeybees

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In silico methods, such as quantitative structure activity relationships (QSARs) are mostly limited to predicting acute toxicity values. In this work we aim to predict chronic toxicity values using the interspecies correlation estimation (ICE) method. However, we applied the existing ICE methodology within and not between species, hence as intraspecies and not interspecies models.

To develop the models, regulatory acute and chronic toxicity data on insecticides were collected for water fleas (*Daphnia magna*; OECD TG 202 and 211), non-biting midges (*Chironomus* sp.; OECD TG 235 and 219) and honeybees (*Apis mellifera*; OECD TG 213/214 and 245) from publicly available regulatory documents and databases. These data are typically of high quality and derived often from GLP studies following OECD testing guidelines. Following the methodology described in the US EPA's Web-ICE manual (V4.0) only bound values were retained. The statistical correlation of log-transformed toxicity values was analyzed by Spearman's test. Thereafter, additional (statistical) criteria from the Web-ICE manual were applied for selecting robust ICE models.

We identified robust acute-to-chronic intraspecies correlation estimation models for daphnids, chironomids, and two for honeybees: one on basis of the contact and one on basis of the oral test. The chronic bee test in the robust ICEs was the 10-d adult honeybee test (for the larval chronic test, no robust intraspecies correlation models were identified).

So, with the identified robust intraspecies models for insecticides, we can predict the chronic outcome, i.e., NOEC or NOEDD, on basis of the acute test endpoint, i.e., EC50 or LD50. For honeybees it has the additional advantage that prediction of endpoints can be done all year round, as opposed to the testing which is seasonally limited. The developed intraspecies models can be used in addition to other in silico tools such as QSARs and

interspecies estimation models to obtain a more comprehensive toxicity profile of insecticides in early stages of development.

1.01.P-Mo013 Uncovering Early Transcriptomic Biomarkers of Toxicity in Zebrafish Embryos Exposed to Chemical Mixtures

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Zebrafish (*Danio rerio*) is widely used in toxicology as it combines vertebrate biological complexity with highthroughput practicality. Its human-like genome, rapid development, and transparent embryos allow assessment of chemical effects across biological scales. These features make zebrafish embryos particularly suitable for detecting early transcriptional changes that occur before visible toxic effects appear. However, despite the growing number of studies, the interpretation of chemical-induced transcriptomic changes remains fragmented across compounds and experimental settings, limiting the identification of consistent molecular signatures and hindering predictions of mixture toxicity.

To identify transcriptome-level biomarkers linked to chemical contaminants exposure, a comprehensive data collection was conducted to compile RNA sequencing datasets from zebrafish embryos exposed during the first five days post-fertilization. Seventy-nine studies were selected from public repositories, selecting 2739 datasets involving 201 compounds. These datasets were standardized for gene annotation and classified based on compound class, physicochemical properties, concentration, exposure duration, developmental stage, and mode of action. The Association Plot (APL) tool in R is being applied to explore gene–response relationships across exposure categories, providing an integrative view of transcriptional patterns.

We hypothesize that early-life exposure to diverse chemicals triggers specific transcriptomic responses in zebrafish embryos, involving genes related to developmental signalling, energy metabolism, and cellular homeostasis that may serve as early biomarkers of chemical stress. Preliminary results reveal comparable transcriptional trends across contaminants, with shared biomarker candidates such as *cyp2k18*, linked to xenobiotic metabolism, and *angptl3*, involved in lipid homeostasis and thyroid/steroid hormone regulation related to endocrine-disrupting compounds. Moreover, certain genes (e.g., *cyp19a1b*) show consistent associations across experiments regardless of concentration or exposure windows like in Bisphenol A and other estrogenic compounds datasets, validating *cyp19a1b* as maker of estrogenicity. These transcriptomic patterns offer valuable insights for assessing the combined effects of chemical mixtures and may support future efforts to predict mixture toxicity in aquatic ecosystems.

Disclaimer/Disclosure:

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1.01.P-Mo014 Fish Reproductive Toxicity Testing: From Historical In Vivo Data to Alternative Cell-Based Predictions

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Chemical safety evaluation serves to safeguard ecosystems while enabling society to utilize the beneficial applications of chemical compounds. Conventional assessments remain animal-based, where animals, such as fish, are commonly sacrificed in studies to characterize chemical toxicity. Such approaches present both ethical challenges and practical limitations—they cannot match the pace of chemical innovation or provide sufficient mechanistic insight for the thousands of substances requiring evaluation. Alternatives to these tests are therefore urgently needed. Our aim is to establish an integrated assessment framework utilizing immortalized fish cell lines coupled with predictive computational approaches, enabling chemical reproductive toxicity evaluation without animal testing. Building on our prior work showing that Rainbow trout (*Oncorhynchus mykiss*) cell cultures demonstrate capacity to predict impacts on fish survival and growth, we extended this methodology through the CRACK-IT challenge – SAFE: Innovative Safety Assessment of Fish adverse Effects – supported by the UK NC3Rs, to address adult fish reproductive toxicity. Initially, we constructed a multistage

computational pipeline identifying critical molecular biomarkers underlying fish reproductive toxicity. We tested these biomarkers in fish cell lines through systematic exposure to known reproductive toxicants, negative control chemicals, and compounds with ambiguous toxicity profiles, establishing gene expression profiles. We subsequently compiled all publicly available data on reproductive endpoints, test chemicals, and gene expression patterns into a comprehensive reproductive toxicity database for adult fish. Through machine learning approaches, we leveraged existing in vivo gene expression and chemical data to predict reproductive endpoints, particularly fecundity. Additionally, we investigated potential methodologies for predicting chemical reproductive toxicity from in vitro gene expression patterns. The compiled database—integrating information from ECOTOX, EnviroTox, and the scientific literature—serves as a comprehensive, publicly accessible resource consolidating current knowledge on adult fish reproductive toxicity. It establishes a solid foundation for the development of alternative testing strategies, advances mechanistic insight into reproductive toxicity, and contributes to the 3Rs principles by reducing dependence on animal studies.

1.01.P-Mo015 Beyond Regulatory Testing: Data-driven Mechanistic Insights into the Effects of the Anti-Diabetic Pharmaceutical Metformin in Fish

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Metformin, one of the most widely prescribed anti-diabetic medicines globally, is routinely detected in aquatic environments, prompting concerns regarding its potential effects on fish. Despite extensive research activity, interpretation of metformin's ecotoxicological significance remains challenging due to heterogeneous study designs, endpoints, and reporting practices. Here, we present the first comprehensive, data-driven meta-analysis of metformin effects in fish, synthesising 1,375 datapoints from 28 peer-reviewed studies involving over 14,000 fish. We quantify effect size, direction, and statistical significance across key endpoints including survival, growth, reproduction, and hormone biomarkers, and evaluate concentration–effect concordance.

Across the dataset, 65% of all observations - and 84% of regulatory-relevant observations - showed no statistically significant effect. Reported effect concentrations varied over several orders of magnitude, with limited dose–response agreement between studies. Meta-analysis did not identify consistent adverse effects at concentrations below the regulatory predicted no-effect concentration (1,000 µg/L). While a subset of studies suggested potential reproductive impacts, quantitative synthesis across multiple reproductive endpoints did not support a consistent adverse outcome pathway.

This work illustrates both the challenges and opportunities of integrating non-regulatory data into chemical risk assessment. By combining effect-size quantification, directionality analysis, and transparent reporting of non-significant findings, we provide a scalable and reproducible data-driven framework for strengthening next-generation ecotoxicology and supporting more nuanced regulatory decision-making.

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1.01.P-Mo016 Derivation of Dynamic Energy Budget Parameters of *Heterocypris incongruens* Using High-Throughput, Standardized Ecotoxicological Test Conditions

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One of the major obstacles in the creation of new Dynamic Energy Budget (DEB) models in small aquatic invertebrates is the derivation of parameters associated with endpoints such as growth and reproduction, due to the difficulty of measuring changes in mass over a period of time. Recent advances in high-throughput ecotoxicological tests have demonstrated growth, reproduction and survival can be measured simultaneously, however, they are constrained to *Daphnids* and their application to other aquatic invertebrates has not been proved yet.

Therefore, an experiment was designed to derive DEB parameters in an ostracod species not often used in Environmental Risk Assessment.

To derive the DEB parameters in *Heterocypris incongruens*, individual organisms were purchased as resting eggs

from MicrioBio Tests Inc. (Mariakerke, Ghent, Belgium) and hatched per the provider's instructions at 25°C. They were kept in Elendt M7 media prepared according to OECD D. magna reproduction test and kept in four different incubators at 15, 20, 25 and 30°C with a 16:8 light dark cycle. The *H. incongruens* individuals were deposited into 24 well microtiter plates (83.3922, Sarsted AG & Co, Nurnbrecht, Germany) with one individual per well. At each temperature, organisms were separated into cohorts of 12 individuals and fed six different feeding regimes of *Selenastrum capricornutum* plus Tetra® fish food concentrated at 5g/L, as done in available literature. Organisms were cultured in 2ml of media and changed to a new well plate every four days before scans to remove molts and keep food levels stable.

Survival was tracked every other day, and growth and reproduction was tracked every four days, coinciding with media changes.

Images of individual ostracods in their wells were taken with the Cell Imager Cell3iMager (CC-5000, SCREEN holdings Co. Ltd., Kyoto, Japan) every four days. To ensure an in-focus image was obtained, bracket scans at multiple heights were used.

Parameter derivation will be done with the DEBtool software package.

Preliminary analysis of the data show that temperature has the greatest impact in reproduction and survival, and effects in growth being present at both different temperatures and feeding regimes, with the DEB parameter derivation currently ongoing.

Results of this study could demonstrate the generation of a novel DEB under conditions similar to those of a standardized ecotoxicological test, facilitating the integration of these models into regulation.

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1.02 Beyond Exposure: Epigenetic Mechanisms, Omics Integration, and AI for Long-Term Environmental Impact

1.02.T-01 Parental Exposure to the Toxic Dinoflagellate *Alexandrium minutum* Affects Offspring DNA Methylation in the Pacific Oyster *Magallana gigas*

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Blooms of the toxic dinoflagellate *Alexandrium minutum* are recurrent on the French coasts, sometimes reaching high concentrations ($>10^7$ cells. L⁻¹). This microalgae can produce paralytic shellfish toxins (PSTs) but also uncharacterized bioactive extracellular compounds (BECs), often associated with harmful effects on marine organisms. The Pacific oyster *Magallana* (=Crassostrea) *gigas*, an important commercial species, is regularly exposed to *A. minutum* during its reproductive period. Studies demonstrated effects of direct exposure to *A. minutum* on oyster gametes and early life stages. Furthermore, parental exposure during gametogenesis also affects gametes, reducing their quality and fertilization potential, as well as offspring, reducing larval size and survival during settlement. Nevertheless, the molecular mechanisms underlying these intergenerational effects remain unknown. In recent years, epigenetic mechanisms, mainly DNA cytosine methylation, have attracted growing interest due to their role in transmitting life traits across generations. Environmental factors experienced by parents, whether biotic or abiotic, can modify DNA methylation, which in turn may shape phenotypic plasticity of offspring. During gametogenesis in mammals, DNA methylation undergoes reprogramming in both female and male gametes. Different timing of stress exposure could therefore imprint distinct DNA methylation marks in the germline, potentially transmitted to subsequent generations. In this study, we exposed a first generation of adult oysters (F0) to *A. minutum* (AM89BM strain) during gametogenesis: either at the initiation or the mature stage. We monitored larval development and settlement, and sequenced spat methylomes (F1), targeting 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) modifications. While no major effects were observed at the phenotypic level in adults and their offspring, 5mC and 5hmC showed differences in the overall variance of methylation levels between conditions. Spats from exposed or non-exposed broodstock displayed differential methylation at several cytosine locations. Most of them belonged to genes related to the structural constituent of chromatin and nucleosomes, involved in genome reorganization. These epigenetic modifications may contribute to genomic flexibility and thereby participate in allowing organisms to adjust to their changing environment.

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1.02.T-02 Multigenerational exposure and epigenetic regulation in duckweed (*L. minor*) under chronic radiation: non-standard responses informing future AOPs

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Chronic, multi- and transgenerational exposure to ionizing radiation remains insufficiently represented in current environmental risk frameworks, which are largely derived from acute laboratory studies. To address this gap, we investigated long-term stress responses in the freshwater macrophyte *Lemna minor*, a model organism with rapid clonal propagation enabling multi-generational assessment. Plants were exposed for six weeks (~19 generations) to external gamma radiation (0.5–480 mGy/h), Sr-90 (40–4,000 Bq/L), Cs-137 (40–40,000 Bq/L), or combined radionuclide treatments. Weekly subsampling enabled kinetic analyses of growth, photosynthesis, antioxidative enzyme activity, transcriptomics, and epigenetic regulation, including DNA methylation via whole genome bisulphite sequencing.

Results revealed dose- and time-dependent responses across all exposure scenarios. Growth showed transient hormetic effects, while biochemical and molecular endpoints indicated acclimation and adaptation processes. Stress responses differed by radiation type: gamma exposure induced persistent transgenerational effects maintained even after recovery, supported by gene expression changes linked to DNA methylation. Sr-90 exposure triggered early antioxidant responses and promoted epigenetic machinery at later stages, whereas Cs-137 showed enhanced photosynthetic activity under comparable dose rates indicating plant coping mechanisms. These divergent mechanisms highlight radiation-type specific pathways of plant stress adaptation. The consistent underlying stress responses observed across generations suggest potential destabilization of populations under chronic exposure, underscoring the need to incorporate long-term and epigenetic endpoints into quantitative Adverse Outcome Pathways (AOPs). Our findings will contribute to refining plant AOPs in the AOPwiki, extending current frameworks beyond acute exposure scenarios to better support environmental radiation protection.

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1.02.T-03 Decoding Neurobehavioral Disruption: Multi-omics Insights in Cadmium and Ciprofloxacin-Exposed Seabass

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Cadmium (Cd) and ciprofloxacin (CIP) are emerging aquatic contaminants with neurotoxic potential. Cd, a trace metal from industrial and agricultural sources, and CIP, a fluoroquinolone antibiotic resistant to water treatment, can induce oxidative stress and disrupt neuronal functions. This study assessed their neurotoxicity in juvenile seabass (*Dicentrarchus labrax*) following 21-day dietary exposure to environmentally relevant concentrations. Fish were fed diets containing Cd (100 or 10 mg·kg⁻¹) or CIP (10 or 1 mg·kg⁻¹). Brain samples were analyzed for epigenetic (DNA methylation), transcriptomic (RNA-seq), antioxidant defenses, oxidative damage, neurotransmission (acetylcholinesterase activity), and behavior. Both contaminants reduced brain glutathione levels, increased AChE activity, and altered locomotor behavior, including reduced swimming activity, increased freezing episodes, and higher social cohesion—indicative of anxiety-like responses. Oxidative stress markers showed treatment-specific patterns: Cd increased SOD activity and lipid peroxidation, while CIP caused glutathione depletion without major changes in SOD. These findings suggest Cd and CIP impair neural signaling and antioxidant balance, leading to behavioral dysfunction that may compromise ecological fitness. Epigenetic and transcriptomic analyses are underway to link molecular alterations with observed phenotypes,

highlighting the need for integrating advanced approaches to assess subtle neurotoxic effects of contaminants in aquatic organisms.

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1.02.T-04 Epigenetic Insights from DNA Adductomics: Early Indicators of Environmental Stress?

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High-resolution DNA adductomics offers a powerful approach to detect early molecular alterations induced by environmental contaminants, yet its application in ecotoxicology remains limited. Here, we established an HRMS-based, non-target DNA adductomics workflow to characterize environmentally induced DNA modifications in *Gammarus pulex* and applied it to evaluate temporal and spatial patterns upstream and downstream of a wastewater treatment plant (WWTP) between 2021 and 2025.

DNA was enzymatically digested and analyzed using LC-HRMS with non-target acquisition, automated MS/MS fragmentation, and in-silico fragmentation-based molecular annotation. In parallel, water samples were screened for ~50 contaminants, including pesticides and pharmaceuticals. The WWTP underwent major upgrades during the study period, a biological upgrade in 2023 and an activated-carbon-based chemical treatment upgrade between 2023 and 2024.

The adductomics workflow detected several hundred DNA adducts across individuals, covering diverse chemical classes and biological processes. Approximately 15 statistically significant features were selected for Partial Least Squares Discriminant Analysis (PLS-DA), providing strong discriminatory power for identifying exposure-related patterns. The multi-year dataset revealed substantial annual variability, with the most pronounced upstream–downstream differences observed in 2025. Downstream organisms exhibited elevated overall adduct burdens, driven particularly by increases in epigenetically relevant adducts such as methylated and hydroxymethylated nucleosides. Although activated carbon treatment reduced the general chemical burden in the water column, a heavy stormwater overflow event occurred during organism collection, and this short-term disturbance was not fully captured by routine chemical monitoring. In contrast, the adductome profiles may reflect this episodic exposure, indicating that DNA adductomics could be sensitive to complex and transient environmental influences.

Overall, this work demonstrates that non-target DNA adductomics provides early, mechanistically interpretable molecular endpoints capable of resolving subtle exposure differences that conventional chemical analysis may miss. By linking environmental gradients to molecular damage and epigenetic alterations, DNA adductomics supports next-generation environmental risk assessment, particularly in systems influenced by multiple and dynamic stressors.

1.02.T-05 Detection of transcriptomic biomarkers for environmental stressors in *Paracentrotus lividus* embryos using machine learning approaches

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The sea urchin *Paracentrotus lividus* provides a robust model for studying the effects of environmental stressors. *P. lividus* embryos were exposed to the pharmaceutical citalopram (CIT) and the biocide triclosan (TC) within a global change scenario that included elevated temperature and sea water acidification, using a factorial design. Transcriptomic profiles were generated by RNAseq and analyzed using a sparse Partial Least Squares Discriminant Analysis (sPLS DA) model reinforced with bootstrapping to identify genes that discriminate each experimental condition.

Machine learning (ML) techniques proved useful in analyzing RNA-seq data from complex experimental designs, and allowed the identification of discriminant biomarkers with specific stressor-dependent expression

patterns. The sPLS-DA model identified 42 biomarkers for thermal stress, 24 biomarkers for CIT exposure and 15 biomarkers for TC exposure. Furthermore, specific biomarker sets associated with the interactions between global change stressors and contaminants were identified. Specific primers were designed and used in qRT PCR to validate the biomarker discovery tool.

The increasing accessibility of omics analyses, in terms of both methodology and cost, is driving a rise in the complexity of data analysis and interpretation. In ecotoxicology, RNAseq analysis has paved the way for performing omic factorial experiments, which are essential for investigating the interactions between multiple environmental conditions and contaminants, an ecologically relevant strategy crucial for addressing current and future contamination scenarios. This methodology identifies more robust biomarkers than classical differential expression analyses, prioritizing those with high exclusivity for a particular experimental condition. Although this study focused on two contaminants and two stressors, the methodology is scalable to include additional treatments and replicates, which would yield more robust discriminant biomarkers and enable the model's predictive capacity for class assignment.

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1.02.P Beyond Exposure: Epigenetic Mechanisms, Omics Integration, and AI for Long-Term Environmental Impact

1.02.P-We001 Exposure to Tetrabromobisphenol A Reprograms Hepatic Lipid Metabolism Both in Mothers and Their Offspring

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Tetrabromobisphenol A (TBBPA) is one of the most used brominated flame retardants, and its environmental exposure has raised extensive attention about potential health hazards. Previous studies have suggested its lipid metabolism toxicity, but its effect on mothers and offspring and the underlying mechanism remains unclear. In this study, TBBPA concentrations in serum from mice exposed to different doses and 971 mid-pregnancy women were detected via HPLC-LTQ-MS to determine an environmentally relevant dose. C57BL/6J mice were then subjected to daily TBBPA exposure (0.2 mg/kg-bw/day) during pregnancy and lactation. Serum biochemistry and multi-tissue H&E staining (liver/adipose/intestine/placenta) were used to assess systemic lipid metabolism in dams and offspring. For mechanism analysis, liver transcriptome, histone modifications (H3K4me3/H3K27ac) and RNA polymerase (RNAPolII), as well as metabolome were detected via RNA-seq, CUT&Tag, and LC-MS, respectively. The results showed that mid-pregnancy (GD12.5) was a maternal sensitive window, with increased fasting blood glucose, liver/adipose coefficients, hepatic lipid deposition/inflammation, reduced free fatty acids, and FoxO pathway enrichment (downregulated *Mfsd2a/Acot2*) indicating lipid reprogramming; while offspring only displayed mild hepatic inflammation with sex-specific lipid metabolism patterns. Epigenetically, offspring had abnormal hepatic H3K4me3/SETD4 levels, with redistribution of H3K4me3/H3K27ac/RNAPolII at lipid metabolism-related gene promoters binding FOX factors. These findings demonstrate environmentally relevant maternal TBBPA exposure disrupts maternal lipid metabolism via FoxO signaling and induces sex-specific offspring metabolic perturbations through SETD4-mediated H3K4me3 remodeling. This work provides a scientific basis for TBBPA safety limits, highlights gestational/lactational exposure management, and advances understanding of early-life chemical-induced metabolic reprogramming via epigenetics.

1.02.P-We002 The transcriptome and epigenome of the marine calanoid copepod *Acartia clausi* after short-term cadmium exposure

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Recent evidence indicates that environmentally driven epigenetic modifications can exert persistent effects on gene transcription across multiple generations. These findings underscore the importance of examining the consequences of pollutants to better understand their long-term impact on populations. 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 phenotypic endpoints, (behaviour and enzymatic) DNA methylation and transcriptome wide changes 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 zooplankton species with a key role in pelagic systems and widely recognized as a model organism in ecotoxicology. Oxidative stress and damage were evaluated by measuring glutathione S-transferase (GST) activity and lipid peroxidation using the TBARS assay, while genotoxicity was assessed through DNA damage analysis with the comet assay. Post-exposure swimming behavior of *A. tonsa* was further examined using automated video tracking. Epigenetic changes were assessed through quantification of global 5-mC DNA methylation and whole-genome bisulfite sequencing (WGBS). We also sequenced the transcriptome and studied differences in the copepod's gene expression as the molecular processes underpinning the studied phenotypic effects. Global 5-mC DNA methylation levels showed a slight decrease in exposed copepods, indicating a trend toward global hypomethylation. Phenotypically, this was associated with a marked rise in genotoxicity, a significant increase in GST activity, and elevated oxidative damage. From the obtained gene expression data, we aimed to identify the most active metabolic pathways and we tried to place these results into a broader context of observed physiological activities. Next, we constructed gene-co-expression networks, identified hub genes and we tried to obtain a mathematical relationship between these networks/hub genes and the phenotypic characteristics of interest. As such, we aim to identify epigenetic mechanisms that can be consistently anchored to phenotypic changes and to provide a molecular basis for adverse outcome pathways for toxicity caused by stressors such as cadmium in copepods.

1.02.P-We003 Integrating Transcriptomic Insights to Predict Long-Term Impacts of Emerging Contaminants Under Climate Stressors

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Pharmaceuticals and personal care products (PPCPs), such as citalopram (CIT) and triclosan (TC), are increasingly detected in marine ecosystems at environmentally relevant concentrations, raising concerns about their ecological impact. Concurrently, climate change drivers such as ocean warming (OW) and ocean acidification (OA) may interact with chemical stressors, producing complex biological responses. This study assessed phenotypic and transcriptomic effects of CIT and TC, alone and combined with OW and OA, on larvae of the sea urchin *Paracentrotus lividus*, a sentinel species in ecotoxicology.

Fertilized eggs were exposed for 48 h post-fertilization to EC₁₀ concentrations of CIT (46.70 µg/mL) and TC (98.92 µg/mL), under control and climate change scenarios (OA: +126 µmol/kg DIC; OW: 24 °C). Growth endpoints were measured at the pluteus stage, and RNA-seq analysis identified differentially expressed genes ($|\log_2FC| \geq 1.5$; $p_{adj} \leq 0.01$) and enriched pathways.

Phenotypically, OA intensified CIT and TC toxicity, reducing larval growth by up to 50%, whereas OW mitigated or reversed these effects. Combined OW+OA conditions neutralized growth alterations, suggesting non-linear interactions. Transcriptomic profiles revealed substantial molecular perturbations even when morphology remained unaffected. CIT alone dysregulated genes linked to metabolism, development, and apoptosis; OA alone had minimal impact, while OW altered transcriptional regulation and morphogenesis. CIT combined with OA or OW triggered broader responses involving energy metabolism, stress signaling, and transcriptional control. TC exposure disrupted cilium assembly, glutathione metabolism, and cytoskeletal organization, with complexity increasing under OW and OA. Multi-stressor scenarios produced the most complex profiles, affecting pathways related to oxidative stress, protein metabolism, and calcium signaling, indicating compromised homeostasis.

These findings highlight that low-level PPCP contamination can interact with climate stressors to elicit significant molecular effects, challenging single-stressor risk assessment paradigms. Omics biomarkers emerge as sensitive tools for early detection of sublethal impacts, advocating for their integration into predictive models. Future research should explore cross-taxa patterns and long-term ecological consequences to inform adaptive management strategies under accelerating anthropogenic pressures.

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1.02.P-We004 Integrative Analysis of Physiological, Epigenomic, and Transcriptomic Changes in *Phaeodactylum tricornutum* Exposed to Cadmium and Ciprofloxacin

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In recent years, epigenetic research has demonstrated that mechanisms such as DNA methylation and histone modifications play pivotal roles in regulating gene expression and mediating environmental adaptation in several microalgae. However, surprisingly few studies have investigated how environmentally driven epigenetic modifications affect diatom physiology and stress-response. To address this gap, we investigated physiological, transcriptomic, and epigenomic changes in the marine pelagic diatom *Phaeodactylum tricornutum*, a model organism in ecotoxicology, following sustained exposure (96 h) to either cadmium (0.293 mg L⁻¹) or ciprofloxacin (0.202 mg L⁻¹), concentrations corresponding to pre-estimated EC₂₀ values based on growth inhibition. Both pollutants were selected due to their documented potential to induce epigenomic alterations: cadmium, a non-essential trace metal, is persistent and bioaccumulative, while ciprofloxacin, a fluoroquinolone antibiotic, has been shown to inhibit histone demethylases and alter methylation patterns in mammalian systems. Oxidative stress and damage were assessed via glutathione S-transferase (GST) activity and lipid peroxidation (TBARS assay), while genotoxicity was evaluated using the comet assay. Epigenetic changes were quantified through global 5-mC DNA methylation analysis and whole-genome bisulfite sequencing (WGBS). Transcriptomic profiling was performed to identify differentially expressed genes and enriched metabolic pathways, which were integrated with the obtained physiological data to elucidate molecular mechanisms underlying observed phenotypic effects. Gene co-expression networks were constructed to identify hub genes and explore their relationships with stress-related traits.

Our results revealed a significant increase in global 5-mC DNA methylation in cadmium-exposed cells, indicating a trend toward hypermethylation, whereas ciprofloxacin exposure did not induce significant changes at the global level. In the transcriptomic analysis we focused on interpreting changes in the key pathways involved in metal detoxification, oxidative stress response, and chromatin remodeling. Network analysis identified candidate hub genes potentially linking epigenetic regulation to the stress phenotypes. These findings provide novel insights into the epigenetic basis of diatom stress responses and contribute to the development of molecular frameworks for adverse outcome pathways (AOPs) relevant to marine ecotoxicology.

1.03.A Bridging Innovation and Interpretation: New Approach Methodologies (NAMs) and 3R Alternatives in Environmental Toxicology

1.03.A.T-01 Adaptation of fish hepatocytes improves the cytotoxicity assessments of substances requiring biotransformation: The curious case of allyl alcohol

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The 96-hour acute fish toxicity (AFT) test is required for chemical hazard assessment, however ethical concerns drive efforts toward implementing alternatives that support the 3Rs (replace, reduce, refine). In 2021, the OECD adopted a fish cytotoxicity test using *Oncorhynchus mykiss* gill cells (RTgill-W1), promoted as a promising alternative to AFT. Despite this progress, full replacement remains difficult for chemicals that require metabolic activation. Allyl alcohol is a notable example, as it must be biotransformed into the toxic metabolite acrolein to induce cytotoxicity. Limited toxicity in RTgill-W1 has been linked to insufficient metabolic enzyme expression, including *aldh8a*, a limitation also observed in zebrafish embryos. Thus, there is a need for metabolically competent in vitro models that can reliably assess such substances. Primary hepatocytes (HEP) show high metabolic capacity and therefore may enable more accurate assessment of metabolically activated chemicals. While commonly used in in vitro bioaccumulation studies (e.g., OECD 319A), their broader potential for acute ecotoxicity remains underexplored. Here, we evaluated metabolic activation and cytotoxicity in rainbow trout HEP exposed to allyl alcohol and its metabolite acrolein. Hepatocytes were isolated using a two-step perfusion method, and incubated at 15 °C for 24 or 48 h with 0.03–100 mg/L allyl alcohol or 0.3–50 mg/L acrolein in 24-well plates (500,000 cells/mL). Cytotoxicity was assessed following OECD TG 249 using three endpoints: cell membrane integrity (CFDA-AM), metabolic activity (Alamar Blue), and lysosomal stability (Neutral Red). Media were collected post-exposure for chemical analysis, and EC₅₀ values were determined using log-linear regression. Hepatocytes exhibited concentration-dependent toxicity for both chemicals across all endpoints and exposure durations. Toxicity profiles were highly similar, with allyl alcohol only slightly more potent; EC₅₀

values differed by less than seven-fold. Sensitivity to allyl alcohol was ~1000-fold greater than reported for zebrafish embryos and comparable to 96-h acute toxicity values in the EPA fathead minnow database. These findings suggest that hepatocytes biotransform allyl alcohol into acrolein within 24 h. Overall, rainbow trout HEP represent a metabolically competent *in vitro* model for acute toxicity and provide a valuable complement to RTgill-W1, supporting future reduction and potential replacement of AFT testing.

1.03.A.T-02 Cellular Diversity Behind Ecotoxicogenomics: Single-Cell and In Vitro Insights into Mycophenolic Acid Exposure in Rainbow Trout Gills

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Pharmaceuticals pose unique ecotoxicological assessment challenges, being designed to elicit specific receptor-mediated effects. RNA sequencing is therefore an appropriate tool for environmental risk assessment (ERA), revealing both pharmacological mode of action (MoA) and off-target effects. The growing list of active pharmaceutical ingredients makes vertebrate testing ethically questionable, driving adoption of animal alternatives such as the rainbow trout epithelial gill cell line RTgill-W1. Combining cell culture with transcriptomics can generate mechanistic insights more ethically, but systematic differences between *in vivo* and *in vitro* models (particularly gene expression profiles) must be considered. Importantly, the RTgill-W1 culture is a single cell type, and an intact gill contains a range of cells with varied function.

The work presented is a case study of the immunosuppressant drug mycophenolic acid (MPA) both *in vivo* (rainbow trout gill) and *in vitro* (RTgill-W1). MPA functions by binding to inosine-5'-monophosphate dehydrogenase (IMPD) and inhibiting purine synthesis. This disproportionately depletes T and B cells, which (unlike other cell types) rely on *de novo* purine synthesis and cannot switch to alternative synthesis pathways. Given that MPA targets immune cells specifically, single cell sequencing analysis was employed to evaluate the diversity of cells within a rainbow trout gill. This data revealed 20 cell types within the rainbow trout gill tissue: including a lymphocyte population which is key to the MPA MoA. Here it was hypothesised that the unicellular RTgill-W1 cell line will not accurately represent the predicted immunosuppressant effect of MPA *in vivo*.

In the *in vivo* results, the expression of immune system related genes was significantly down regulated in all exposure conditions, which may make the fish more vulnerable to other stressors in aquatic environments. This immunosuppressant effect was notably not observed in the *in vitro* results, however developmental genes were suppressed in both the cultures and the intact gills at higher MPA concentrations. This cell cycle interference could reduce the gills capacity for growth and structural remodelling, which is important for its role as an environmental protective barrier for fish. Our case study exposure reveals the necessity to consider the greater complexity of the cell types contained in the intact gill when considering the utility of cell lines, such as RTgill-W1, in ERA.

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1.03.A.T-03 Risk-based prioritization of persistent and mobile chemicals using new approach methodologies (NAMs)

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Persistent and mobile chemicals are of growing concern as they can accumulate and travel large distances from their point of emission. These chemicals can pollute water resources that are used for the production of drinking water. For many persistent and mobile chemicals used and produced in Europe, no or limited data is available on toxicity. As traditional *in vivo* toxicity testing raises concerns regarding limited testing capacity, high costs and animal welfare, novel strategies are required to prioritize chemicals for further toxicity testing, or for efficiently allocating funds for prevention or mitigation efforts.

Within the ZeroPM project, we have developed a low-tier risk-based prioritization matrix using *in vitro* and *in silico* new approach methodologies (NAMs). In this matrix, the combined estimations of exposure and hazard

are used to determine the priority of each chemical. The exposure estimate is based on measured groundwater concentrations calculated to free human plasma concentrations using physiology-based kinetic models, reflecting a worst-case exposure scenario. The hazard is estimated by testing the chemical in in vitro bioassays and deriving benchmark concentrations which were calculated to free medium concentrations using a virtual in vitro distribution model. As a proof-of-concept, the matrix was used to prioritize 9 triazine, 16 triazole and 11 PFAS compounds classified as persistent and mobile. Given the data-richness of these compounds, the predictive value of the NAMs could be evaluated with toxicokinetic and toxicodynamic in vivo data. Based on the known toxicity of the test chemicals, the in vitro methods focused on endocrine disrupting properties, liver toxicity and developmental toxicity.

In general, the hazard estimates based on in vitro data were more conservative than based on in vivo data. When comparing toxicological benchmark concentrations to exposure estimates, PFOA was the only tested high priority chemical. PFOS and PFHxS were prioritized after PFOA, based on lower exposure concentrations, that were <1% of the toxicological benchmark concentration. Chemicals as difenoconazole and fenbuconazole had similar ranges of toxicological benchmark concentrations as PFOS and PFHxS, but were categorized as lower priority chemicals due to their lower exposure estimate. This study demonstrated the applicability of a NAM-based prioritization matrix, but also highlighted the importance of accurate exposure estimations.

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1.03.A.T-04 Next Generation Hazard Assessment of Legacy and Alternative Phthalate Plasticizers Using New Approach Methodologies within an Integrated Approaches to Testing and Assessment (IATA) Framework

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Regulatory restrictions on legacy phthalates have driven the introduction of alternative plasticizers; however, their safety profiles remain poorly characterized. Combined with reduced animal testing, this underscores the need for structured frameworks integrating diverse mechanistic evidence to evaluate data-poor chemicals efficiently. This study applied an Integrated Approaches to Testing and Assessment (IATA) framework to comparatively evaluate developmental and reproductive toxicity of legacy and alternative phthalates, assessing how multiple New Approach Methodologies (NAMs) can collectively inform hazard potential and reduce uncertainty.

We integrated evidence from regulatory in vivo reports, literature-derived in vitro assays, high-throughput screening from ToxCast, and artificial intelligence-based predictions. These datasets were organized through adverse outcome pathway (AOP) networks related to male reproductive toxicity. Both qualitative evidence mapping and quantitative human-equivalent dose estimation using high-throughput toxicokinetics were conducted, with findings compared to traditional animal study outcomes.

Legacy phthalates exhibited strong and consistent toxicity signals across multiple evidence streams, whereas alternatives showed weaker or inconsistent activity, reflecting lower hazard potential and substantial data gaps. Quantitative comparison demonstrated that NAMs-derived doses can approximate traditional reference values but remain limited by metabolic coverage gaps and incomplete endpoint representation.

This study demonstrates feasibility of NAMs-informed IATA for comparative phthalate assessment. For data-rich legacy compounds, NAMs provided complementary mechanistic information, reducing uncertainty; for data-poor alternatives, NAMs identified signals guiding prioritization and precautionary actions. The approach underscores the importance of integrating methods within AOP frameworks under FAIR principles and developing tiered testing strategies. Continued expansion of mechanistic datasets and refinement of extrapolation models will strengthen hazard evaluation for chemical substitution and regulatory decision making.

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1.03.A.T-05 European Seabass (*Dicentrarchus labrax*) Hepatic Spheroids: a Novel in Vitro Model to Decipher Biotransformation and Mechanisms of Action of Chemical Contaminants

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The marine chemical exposome encompasses thousands of legacy and emerging contaminants, along with their transformation products, whose toxicity, modes of action and biotransformation remain only partially characterised in marine organisms. In this context, in vitro New Approach Methodologies (NAMs) are increasingly promoted to generate mechanistic data while reducing dependence on in vivo testing. Among these, three-dimensional (3D) liver models such as hepatic spheroids are gaining attention thanks to their better conservation of tissue architecture and metabolic functions than conventional two-dimensional (2D) monolayers, making them particularly suitable for studying chemical contaminant biotransformation and mode of action. In this study, the aim was to develop and characterise a 3D hepatic model from primary liver cells of European seabass (*Dicentrarchus labrax*), a marine teleost of high ecological and economic importance. Hepatic spheroids were generated and benchmarked against both the native liver and classical 2D hepatic cultures. The model was characterised using biometry, cell viability, histology and expression of key liver genes. Seabass hepatic spheroids remained viable over more than a month and retained more pronounced hepatic features than 2D cultures, supporting a more stable and liver-like phenotype. To evaluate their functional relevance for chemical contaminant fate and effects, spheroids were exposed to the model polycyclic aromatic hydrocarbon pyrene. Biotransformation capacity was demonstrated in both 2D and 3D cultures, including activation of Phase I and II metabolism. Ongoing proteomic analyses are further refining the phenotype of seabass hepatic spheroids relative to the parent organ and 2D cultures, and are being used to investigate pyrene modes of action across culture formats. Overall, our results support this 3D hepatic model as a species-relevant in vitro system. The ability to generate more than 70,000 spheroids from a single fish liver highlights its potential for higher-throughput toxicological analyses and a substantial reduction in the number of animals required in marine ecotoxicology.

1.03.B Bridging Innovation and Interpretation: New Approach Methodologies (NAMs) and 3R Alternatives in Environmental Toxicology

1.03.B.T-01 Mechanistic Insights into 6PPD-Quinone Toxicity using Brain- and Gill-derived Rainbow Trout (*Oncorhynchus mykiss*) Cell Lines as New Approach Methodologies (NAMs)

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6PPD-quinone (6PPD-Q), formed through the environmental oxidation of the tire antioxidant 6PPD, has been identified as the primary driver of urban runoff mortality syndrome (URMS), responsible for the mass mortality of Coho salmon. However, the cellular and molecular pathways underlying its toxicity remain poorly characterized. To address this knowledge gap, we employed our well-established cell-based toolbox, consisting of rainbow trout (*Oncorhynchus mykiss*) cell lines derived from different tissues and serving as relevant New Approach Methodologies (NAMs). In vitro cytotoxicity assays (OECD TG 249) revealed tissue-specific effects: 6PPD-Q was highly toxic to RTbrain cells but not to RTgill-W1 cells, derived from brain and gill tissue, respectively. Surprisingly, IPPD-Q - derived from the tire additive IPPD - showed no toxicity in RTbrain cells at the tested concentrations.

To investigate the mechanisms underlying these tissue- and chemical-specific responses, we performed RNA sequencing (Illumina NovaSeq X platform) and global proteomic analysis (nanoLC-MS/MS) after 24 h of exposure. Differentially Expressed Genes (DEGs), identified via DESeq2, and subsequent Gene Ontology (GO) analysis revealed both shared and tissue specific responses to 6PPD-Q: genotoxic and oxidative stress pathways were upregulated in both cell lines, whereas inflammatory/immune response were uniquely upregulated in RTgill-W1 and neuronal function/ion channel regulation uniquely in RTbrain. Comparison of 6PPD-Q vs IPPD-Q exposure in RTbrain cells showed downregulation of detoxification pathways along with activation of metabolic and stress responses in 6PPD-Q treated cells, potentially explaining its higher toxicity compared to

IPPD-Q. Preliminary proteomic analysis identified several differentially expressed proteins (DEPs), with GO terms broadly consistent with transcriptomic findings. RNA sequencing and global proteomic analysis highlight pathways that may underlie tissue-specific responses and the mechanisms of action of 6PPD-Q. Next steps include validating key genes by RT-qPCR, expanding toxicity endpoints (e.g., ROS formation), and quantifying internal concentrations to assess cellular bioaccumulation. Overall, these results support the growing potential of fish cell lines as NAMs for mechanistic toxicology of tire additives, paving the way toward more predictive and ethically aligned environmental risk assessment of these emerging contaminants.

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1.03.B.T-02 Development of Fish Cell-based Reproductive Toxicity Assays

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Chronic fish toxicity data, which include reproductive toxicity endpoints, are required by regulators such as EFSA, ECHA, and EMA. Tests to obtain these data demand substantial numbers of animals, and time and money. Within the framework of the NC3Rs CRACK IT Challenge “SAFE”, we initiated the development of in fish cell line-based bioassays that can predict chemical impact on fish reproduction.

As the first step in this endeavour, we have developed a computational pipeline to identify 372 potential biomarkers of reproductive toxicity in fish (based on in vivo data) and tested their basal expression in chemically exposed permanent rainbow trout (*Oncorhynchus mykiss*) cells derived from liver and testis (in vitro). In the second step, we exposed the cell lines to four chemicals with known reproductive effects in fish (17 β -estradiol, 17 β -trenbolone, fadrozole, and flutamide) and two chemicals with no known reproductive effects (saccharin and sodium dodecyl sulphate). In the third step, we used the newly generated in vitro data to train machine learning (ML) algorithms. The amount of in vitro data, however, constrained model performance, indicating that more data is required. To temporarily overcome this limitation, we generated synthetic data comprising 34 additional treatments and achieved >70% model accuracy.

This proof-of-concept study demonstrated that (1) measuring biomarkers in cultured fish cells and interpreting the data with ML algorithms is a promising approach for predicting fish reproductive toxicity, and (2) expanding the dataset with approximately 30 additional chemical tests would enhance model performance to a robust and reliable standard, as well as build further confidence in this suite of assays, define their applicability domain, and advance them toward formal validation.

As a next step, we plan to incorporate cell lines derived from other organs, such as the brain and ovary, to achieve broader coverage of tissues involved in reproduction. Ultimately, we envision this work to evolve into a modular in vitro/in silico assay for fish reproductive toxicity, positioned within the framework of the fish invitrome and animal replacement strategies more broadly.

1.03.B.T-03 In Vitro Transcriptomic Points of Departure (tPODs) for 42 diverse chemicals in a human (Caco-2) and fish (RT-Gill) cell line

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Transcriptomic points of departure (tPODs) have emerged as of scientific and regulatory interest, and there is increased interest in advancing the tPODs approach using cell lines. Towards this, we have developed the ‘TPD-seq’ workflow which links microplate-based exposure methods involving cell lines, with a commercially available RNA-seq kit, with a cloud-based bioinformatics tool (ExpressAnalyst.ca). To date the TPD-seq workflow has been applied to study positive/negative control chemicals routinely used in toxicological studies as well as 6PPD-quinone which is a chemical of immediate regulatory concern. To assess the scalability of the TPD-seq workflow, here it was applied to rainbow trout (*Oncorhynchus mykiss*) gill (RTgill-W1) and human intestinal epithelial cells (Caco-2) exposed to 42 chemicals of varying properties. Cells were exposed in a concentration-dependent manner (0-1000 μ M, spaced on a semi-log scale), following which cytotoxic and transcriptomic responses were measured and compared. Bioinformatics analysis yielded tPOD values. For Caco-

2 cells, the number of reads per sample was about 2-42 million, with 80-98% being mapped to genes. The number of differentially expressed genes (DEGs) across the chemicals was in the thousands, with gene BMDs ranging from 10 – 3,271. The tPODmode values ranged from 0.03 - 62 µM. Transcriptomics analysis for the RTgill-W1 cells is ongoing. Thus far, as like Caco-2, two methods for differential expression analysis (DESeq2 versus the Williams trend test) did not significantly affect tPOD results. 3,4-dichloroaniline (DCA) which was used as a positive control across multiple exposures resulted in tPODmode ranging from 0.01 to 0.03 µM (repeated four times). Thus far the TPD-seq workflow is generating cytotoxicity data, tPOD values, and lists of genes and potentially impacted biological pathways for a fish and human cell line. It is interesting to note that molecular/tPOD data can be derived for many chemicals that are not deemed cytotoxic. The derivation of this data was rapid and efficient compared to traditional animal-based methods, while also returning tremendous biological (molecular) information.

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1.03.B.T-04 Evaluating the Protectiveness of High-Throughput Transcriptomics Data from fish cell lines for Environmental Risk Assessment

Claudia Rivetti, Jacob Collins, Geoff Hodges and Bruno Campos, Unilever, United Kingdom

The accelerating pace of chemical innovation necessitates efficient, mechanistically informative approaches for environmental safety assessment. Traditional animal-based toxicity testing is increasingly challenged by ethical, logistical, and scientific limitations, driving the adoption of New Approach Methodologies (NAMs). High-Throughput Transcriptomics (HTTr) offers a promising solution by enabling rapid evaluation of chemical bioactivity in vitro. This study evaluates whether transcriptomic points of departure (tPODs) derived from zebrafish cell lines are protective of apical endpoints observed in aquatic species. Forty-two chemicals were tested in ZFL (liver) and ZEM2S (embryonic fibroblast) cell lines using the TempO-Seq platform and analysed via gene-level and signature-based dose-response modelling. When converted to predicted external water concentrations using quantitative in vitro-in vivo extrapolation (qIVIVE), tPODs were generally protective compared to in vivo effect concentrations curated in the ECOTOX Knowledgebase. Benchmark comparisons revealed that HTTr-derived PoDs demonstrate conservatism with median in vivo values, supporting their utility for screening-level hazard assessment. Furthermore, integration of biological pathway analysis and cross-species susceptibility tools demonstrated the potential for extrapolating chemical effects beyond the tested taxa. These findings underscore HTTr as a scientifically robust and ethically preferable approach for advancing environmental risk assessment, while highlighting the importance of multi-cell-type strategies to capture tissue-specific responses.

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1.03.P Bridging Innovation and Interpretation: New Approach Methodologies (NAMs) and 3R Alternatives in Environmental Toxicology

1.03.P-Tu001 Assessing the environmental safety of microbial pesticides: Opportunities to apply the 3Rs *Amy Marriott and Natalie Burden, NC3Rs, United Kingdom*

Microbial pesticides are an expanding class of crop protection products, increasingly developed for global markets as sustainable alternatives to conventional chemicals. To achieve worldwide registration, environmental data packages must satisfy diverse regulatory requirements across multiple jurisdictions. These requirements often differ in scope and interpretation, leading to duplicative testing and, in some cases, unnecessary vertebrate studies that add limited scientific value. Such practices raise concerns in terms of addressing the 3Rs (Reduction, Refinement and Replacement of animal use) and regulatory efficiency.

Current ecotoxicology test guidelines were designed for chemical substances and are often unsuitable for living

microorganisms, which exhibit unique properties such as infectivity and persistence. This misalignment creates challenges for study design, interpretation, and waiver justification. While some regions allow flexibility, approaches remain inconsistent, and opportunities to apply the 3Rs may not be fully maximised.

This poster will summarise the work the expert NC3Rs Ecotoxicology Working Group are carrying out to scope the global regulatory landscape for microbial pesticide ecotoxicology testing priorities and identify opportunities for better harmonisation and application of the 3Rs. Key opportunities include developing fit-for-purpose guidelines, promoting weight-of-evidence strategies, best practices for study waivers and integrating new approach methodologies (NAMs) to reduce reliance on vertebrate testing. By embedding 3Rs principles into evolving frameworks, the sector can support innovation in microbial pesticide development while maintaining robust environmental protection.

1.03.P-Tu002 Building AOPs for Developmental and Reproductive Toxicity in a 3R Paradigm: PFAS Case Study in *C. elegans*

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A limitation of cell-based in vitro toxicity assays is that they cannot capture downstream key events for systemic toxicity endpoints, as multiple cells and tissues are involved in the adverse outcome. To complement these cell-based assays, *Caenorhabditis elegans* offers a promising whole-organism 3R approach to adverse outcome pathways (AOP) development for toxicity testing. Despite its potential, *C. elegans* has not yet been widely applied to the study of key event relationships (KERs) within AOPs. Per- and polyfluoroalkyl substances (PFAS) represent a timely case study for developing AOPs in the context of developmental and reproductive toxicity (DART) using *C. elegans*.

In this study, we exposed *C. elegans* for 1–6 days to increasing concentrations of two prototypical, data-rich PFAS: perfluorooctanesulfonic acid (PFOS) and perfluorodecanoic acid (PFDA). We assessed upstream and downstream key events associated with DART, including gene expression changes, embryonic lethality, development, brood size and daily reproductive output. Using RNA-seq, expression of DART-related genes was significantly downregulated at low micromolar concentrations, while more downstream key events were perturbed at higher concentrations. When concentration–response relationships were expressed using nominal exposure concentrations, PFDA appeared significantly more potent than PFOS for each measured endpoint. For example, a clear dose–response relationship was observed for total brood size, with EC₅₀ values of 104 µM and 40 µM for PFOS and PFDA, respectively.

However, we also analytically measured internal concentrations of both PFAS in the worms after exposure using LC-MS/MS. When concentration–response relationships were expressed as internal worm concentrations, PFOS and PFDA showed more similar potencies as developmental toxicants, suggesting they act on the same AOP. The internal EC₅₀ was 3 mM for PFOS, while the EC₅₀ for PFDA was 1.5 mM, much higher than the nominal concentrations suggest. These data demonstrate that both PFAS substantially accumulate in *C. elegans* and that PFDA accumulates to a greater extent than PFOS, which can be explained by its higher lipophilicity. Our study demonstrates a clear, similar reproductive toxicity, across biological levels of organisation, following exposure to PFOS and PFDA.

1.03.P-Tu003 Surveying Regulatory Ecotoxicology Practices: Trends and 3Rs Opportunities in Contract Research Organisations

Amy Marriott and Natalie Burden, NC3Rs, United Kingdom

The UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) leads a programme to advance the application of the 3Rs in regulatory ecotoxicology testing. This work is supported by the NC3Rs Ecotoxicology Working Group, which includes experts from regulatory agencies, contract research organisations (CROs), the pharmaceutical, consumer products and (agro)chemical industries, and academia. In collaboration with this group, we conducted a survey of CROs to capture current practices in regulatory ecotoxicology testing and identify opportunities and barriers for implementing 3Rs approaches within standardised test guidelines used for safety assessment.

Building on a previous survey conducted in 2016, this survey examined how in vivo testing trends have evolved, where new approach methodologies (NAMs) are being applied, and where refinement and reduction strategies are already in use. Respondents also highlighted challenges and drivers for wider adoption of existing

3Rs approaches. The findings provide a benchmark for current testing practices and will inform future NC3Rs projects and priorities. By comparing these insights with historical data and tracking changes over time, this work aims to support evidence-based strategies to reduce vertebrate use in ecotoxicology while maintaining robust regulatory decision-making.

1.03.P-Tu004 Exploring Cross-Species Chemical Sensitivity Analysis of Alternative Model Organisms, Using Bayesian Methods, to Evaluate Their Potential to Inform Chemical Hazard Assessment

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Historically, regulatory toxicology has relied on vertebrate species for chemical safety assessment, yet ethical and legislative pressures are accelerating the transition towards non-animal approaches. A major barrier to this shift is that traditional point estimates provide limited insight into uncertainty and hinder robust comparison of sensitivity across taxa. Probabilistic methods such as Bayesian Benchmark Dose (BBD) modelling address these limitations by generating full posterior distributions that characterise both the magnitude and uncertainty of chemical effects.

This study investigates whether BBD distributions can reveal patterns of cross-species chemical sensitivity that may reflect evolutionary relationships. Using immobilisation dose–response data from the PrecisionTox project for five biomedical relevant model species, the analysis will evaluate whether similarities in shape, location, and overlap of posterior distributions are consistent with shared ancestry or divergence between major taxonomic groups. Framing chemical sensitivity as a continuous probabilistic phenotype provides a robust basis for comparative analysis and supports evaluation of alternative model organisms as potential replacements for traditional vertebrate species.

BMD10 posterior distributions were generated for a set of 29 chemicals from the PrecisionTox database tested across all of the five species. These distributions were used to assess cross-species sensitivity rankings, uncertainty, and overlap in responses. Planned analyses will apply phylogenetic reconstruction to the posterior distributions, examining variability across the full species set and comparing reconstructed vertebrate and invertebrate nodes. This will enable assessment of whether inferred toxicity distributions for the ancestral vertebrate lineage differ from or overlap with those of ancestral invertebrates. This approach contributes to the 3Rs and supports broader regulatory use of alternative test species.

Preliminary results show strong agreement in species sensitivity rankings across chemicals, with several vertebrate–invertebrate pairs displaying identical rank orders. Whole organisms, including *C.elegans* and *Daphnia magna*, demonstrated comparatively narrow posterior distributions, suggesting lower uncertainty than human cell assays for the endpoints examined. These findings indicate that distributional phenotypes may capture biologically meaningful similarities across taxa.

Disclaimer/Disclosure:

The dataset used was generated by the PrecisionTox project.

1.03.P-Tu005 Enabling the Next Generation of Avian NAMs: Data Curation, Model Evaluation, and Mechanistic Pathways Forward

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New Approach Methods (NAMs) offer opportunities to reduce animal testing, but their use in avian acute toxicity remains limited due to fragmented data, uncertain predictive performance, and the narrow reliance on a few standardized test species (quail, duck). These domestic models do not capture the physiological and ecological diversity of the ~10,000 extant bird species, raising concerns about the ecological relevance of current assessments. To support the development of more representative, mechanism-informed alternatives, we compiled and evaluated available *in vivo* data and assessed existing computational and experimental approaches.

We curated acute toxicity records from three major databases, obtaining 1,671 high-quality entries for 811 chemicals. Uncertainty analysis showed substantially less experimental noise than in mammalian datasets, providing a strong basis for evaluating NAM performance.

We reviewed Quantitative Structure–Activity Relationship models for avian toxicity; ten models met inclusion

criteria but were constrained by small or undisclosed training sets, limited transparency, and poor sensitivity to highly toxic chemicals. A targeted literature search identified two promising experimental approaches—avian cell cultures and the embryonated egg model—but both suffer from scarce data, lack of standardized protocols, and species-specific variability that further highlights the limitations of using domestic species as universal surrogates.

The literature provided NAM-derived LD₅₀/EC₅₀ values for 72 chemicals, with only two overlapping adult bird LD₅₀ data. Curation also identified a larger DT-40 chicken bursal cell-line dataset (75 chemicals). To explore extrapolation from NAMs to adult birds, we developed a simplified TKTD model using the GUTS framework. Applied to DT-40 cytotoxicity data, predictions were unreliable for neurotoxicants, likely due to missing mechanistic targets. Overall, DT-40 data poorly predicted avian acute toxicity, and hydrophobicity alone did not explain variability.

This work identifies key data gaps and methodological limitations while providing the most comprehensive quality-screened in vivo dataset available. By integrating this resource with systematic evaluation of computational and experimental approaches, we outline a path toward species-appropriate NAM platforms and mechanistically informed TKTD models, supporting reliable and regulatory-ready alternatives to adult bird acute toxicity testing.

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1.03.P-Tu006 High-Throughput Determination of Structure-Activity Relationships in Organophosphate Ester Protein Binding

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Organophosphate esters (OPEs) are a class of contaminants characterized by a pentavalent phosphorous and three ester groups, used commonly as flame retardants, plasticizers, and pesticides. The toxicity of OPEs varies significantly depending on their structural differences (e.g., aryl, alkyl, or halogenated ester side chains). However, structure-dependent toxicity assessments remain limited, largely due to the scarcity of reliable data. Moreover, more than 700 OPEs with diverse chemical structures are currently in production worldwide, underscoring the need for high-throughput approaches to evaluate their structure–activity relationships and potential impacts on human health.

To achieve this, we have synthesized a diverse array of >250 OPEs using ligands from alkyl, aryl, chlorinated, and brominated ester side chains used commonly in OPEs. Syntheses were characterized via 31P NMR and high-resolution mass spectrometry. The protein bindings of the synthetic OPEs were assessed in a high-throughput affinity selection mass spectrometry (AS-MS) assay adapted from our recent study. The synthesized chemicals were incubated with purified protein targets of OPEs before bioactive compounds were pulled out by tagged proteins, washed, eluted, and detected by mass spectrometry. The selected protein targets included FABP1, a recently identified binding target for tri-aryl OPEs with high structural selectivity along with six other nuclear receptors reported to have affinity for OPE binding. Interestingly, when correlating calculated XLogP of synthetic OPEs with FABP1 binding, an inverted U shape structure-activity relationship could be found. Differences in OPE binding affinity between FABP1 and various nuclear receptors will be further investigated to determine whether binding trends persist across different types of proteins. Collectively, this study developed a high-throughput new approach methodology for establishment of structure-activity relationships within a chemical class, which can be further extended to prioritize the most bioactive compounds.

1.03.P-Tu007 Defining Applicability Domains for the OECD 249 Fish Gill Cell Line Assay: Systematic Evaluation of Membrane-active Substances

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As environmental risk assessment still relies heavily on animal testing, new approach methodologies (NAMs) are being developed in line with the principles of the 3Rs (Replacement, Reduction, Refinement). The OECD 249 fish gill cell line assay represents a promising alternative, where rainbow trout RTgill-W1 cells are exposed to test substances for 24 hours. Cell viability is quantified using three fluorescent dyes targeting different metabolic pathways, complemented by visual microscopic evaluation of the cell monolayer.

Our research evaluated membrane-active substances (surfactants) using this approach. While the three fluorescent dyes typically represent distinct cellular metabolic pathways to identify specific MOAs, our

fluorescence data suggested membrane interactions that could have been overinterpreted without complementary analysis. The microscopic evaluation revealed that cells and membranes were clearly damaged, indicating that relying solely on fluorescence data would have led to false positive interpretations of the specific interaction mechanisms. This finding demonstrates the essential role of microscopic assessment in preventing misinterpretation of fluorescence data, particularly for membrane-active substances where cellular damage may confound endpoint-specific signals.

We observed that specific chemical properties lead to distinct cell interactions, explaining previously identified inconsistencies in in vitro to in vivo result correlations across substance groups. These substance specific differences must be systematically identified to define the applicability of the OECD 249 test and its limitations. These findings would provide valuable insights for the regulatory acceptance of alternative methods in environmental risk assessment and could facilitate the transition away from animal testing. Our work contributes to the ongoing refinement of NAMs for more ethical, efficient, and predictive ecotoxicological assessments.

1.03.P-Tu008 Improving Chronic Fish Toxicity Prediction by Fish Early-Life Stage Data analysis and guided development of predictive alternative models

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Chronic fish toxicity data are required by many regulations for environmental risk assessment and hazard classification. Typically, this information is provided from animal tests, mainly the fish early-life stage test (FELST; OECD Test Guideline 210). FELST is one of the most animal-consuming tests in ecotoxicology, with over 23,000 fish used annually in Europe. The LIMIT-FELST project aims to reduce the number and severity of FELST studies through predictive computational and experimental alternatives. We apply a tiered strategy where full concentration–response FELSTs are replaced by single-concentration limit tests informed from computational and experimental models. To develop this approach, we curated a database of FELST studies from public literature, ECHA, and other regulatory dossiers. Each entry was screened for TG210 compliance, and preference was given to studies reporting both no observed (NOEC) and lowest observed effect concentration (LOEC) value for survival and growth. For studies lacking direct survival LOECs, estimates were derived from growth endpoints via regression analysis. This yielded a dataset covering >500 substances. A key step in the data analysis was comparison of effects to the predicted baseline toxicity (i.e., hydrophobicity-driven intrinsic toxicity). We extended an existing baseline toxicity model (Scholz et al. 2018, ETC 37, 955-969) by adding more presumed narcotic compounds and using the dissociation-corrected lipid–water distribution coefficient (D_{lipW}) as descriptor of hydrophobicity. The expanded dataset confirmed the previously established regression for narcotic chemicals, increasing confidence in its use and enabling identification of compounds deviating from baseline toxicity. These were often associated with specific modes of action such as mitochondrial respiration inhibition, kinase signaling interference, endocrine disruption, or redox cycling. We hypothesized that these modes of action can be identified in an alternative testing approach, with similar sensitivity as in the FELST. Therefore, test systems like fish embryo morphology and behavior, cell-based assays, and transcriptomic profiling will be explored to support identification of limit concentrations without effects in full FELSTs. While ultimately the approach aims at a full replacement, the LIMIT approach has the advantage of being compliant with recent regulatory guidelines. The LIMIT approach could reduce animal use by up to 60%.

Disclaimer/Disclosure:

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1.03.P-Tu009 Exploring the Applicability of the OECD TG 249 Fish Cell Line Acute Toxicity Assay in Environmental Hazard and Risk Assessment of Cosmetic Product Ingredients

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The OECD Test Guideline 203 fish acute toxicity test has historically provided data for environmental risk assessment, hazard classification, and regulatory decision-making. However, its reliance on live fish has driven a shift toward alternative methods that align with the 3Rs principles and evolving regulatory expectations. Among these, the OECD TG 249 assay, based on the RTgill-W1 fish gill cell line, represents a key innovation that enables the prediction of acute fish toxicity without the use of vertebrates.

As OECD TG 249 becomes more widely applied, efforts are increasingly focused on refining our understanding of its domain of applicability. Characterizing these boundaries is essential for defining how and when OECD TG 249 can be appropriately used in hazard and safety assessment, screening, and as part of integrated approaches to testing and assessment (IATAs). Seral initiatives are helping to map the OECD TG 249 assay's performance across diverse substance classes, identify conditions where concordance with in vivo outcomes is strongest, and develop guidance for interpretation within regulatory frameworks.

Building on these advancements, the International Collaboration on Cosmetics Safety (ICCS) has initiated a project focused on cosmetic ingredients, a category characterized by high chemical diversity. By carefully choosing test chemicals from diverse functional classes and physicochemical properties, the project seeks to further clarify TG 249's applicability domain. Comparing OECD TG 249 results with existing high-quality in vivo data will seek to evaluate how accurately the in vitro assay predicts toxicity across various chemical classes, whilst also identifying those classes that need further evaluation and consideration. These learnings and the data generated through this comparative assessment can then be incorporated into existing risk assessment frameworks to ensure the appropriate application of this assay in supporting the safe use of cosmetic ingredients in consumer goods.

Disclaimer/Disclosure:

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1.03.P-Tu010 A Next-Generation High-Throughput In Vitro Exposure System for Early Hazard Ranking and Comparative Toxicity Assessment of Cigarette Smoke and Heated Tobacco Aerosols

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Reliability and reproducibility of in vitro toxicological procedures for assessing the hazard potential of complex aerosols are determinant to cope with the innovations in molecular biology. The tools available for simulating exposure of epithelial lung cells are constantly being refined, but there are still no standards – or none have been agreed. This study contributes to close this gap by examining a novel high throughput exposure system that is designed to provide comprehensible exposure data, based on air-liquid interface and continuous flow technology. Using reference cigarette smoke and heated tobacco aerosol emissions, A549 cells grown in 96-well insert microplates were exposed to 11 aerosol concentration levels plus a vehicle control in eight technical replicates each, yielding 96 data points in a single batch. The consistency between nominal aerosol dose metrics and the elicited biological responses (cytotoxicity, genotoxicity, immunomodulation), confirms accuracy and reproducibility. Comparison between both emission classes, supported by chemical analyses, confirmed the transferability of the methodology, providing relative toxicity equivalents that enable an effect-based ranking of tobacco products. The feasibility of high-content approaches such as in situ γ -H2AX (genotoxicity) analysis also indicates an extended scope of application beyond conventional screening purposes. A unique combination of precise aerosol conditioning, uniform dosing and an unprecedentedly low background noise qualifies the high throughput exposure system for providing valuable dose-response data. A high degree of approximation to lung physiology can be derived, which is certainly not a matter of course in the context of continuous-flow exposure, particularly considering the novel microplate format.

1.03.P-Tu011 Contribution of the Ionic Fraction to the Toxicity of Soluble Metallic Nanomaterials

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One of the key challenges in studying the toxicity of partly soluble nanomaterials (NMs) is determining whether the observed toxicity is primarily caused by released ions or by the nanoparticles themselves. Because the solubility of NMs varies with the physicochemical properties of the test medium, and the duration of the experiment, it is important to assess solubility in each specific test.

Therefore, the aim of this study was to investigate the solubility of some metal-based NMs and to assess the contribution of released ions to their overall toxicity.

For this purpose, partly soluble metallic nanomaterials differing in size, shape, and surface coating were investigated: Ag (stabilized with citrate), 10 nm; CuO, 15-20 nm, (PlasmaChem); CuO, <50 nm, (Sigma-Aldrich); Cu, 25 nm; Cu, 50 nm; Cu, 100 nm, (IoLiTec); ZnO NM-110, 42 nm; ZnO NM-111, 34 nm (JRC repository).

NM toxicity was assessed following the OECD Test Guideline No. 249, which evaluates the cytotoxicity in the fish cell line RTgill-W1 applying three assays. The Nanogenotox protocol was used to obtain stable dispersions. Solubility was evaluated following the recommendations of OECD Guidance Document No. 318, using centrifugal ultrafiltration with Amicon 3 kDa filters. Concentrations of the soluble fraction relative to the total NM amount were measured at 0 and 24 h by ICP-MS. In parallel with NM toxicity testing, the toxicity of the corresponding metal ions was assessed by applying their soluble salts (AgNO₃, CuCl₂, ZnSO₄) under identical conditions.

Among the tested nanomaterials, CuO NMs showed the lowest solubility, followed by ZnO, and then Ag and Cu NPs. NMs that are more soluble exhibited greater toxicity. Comparative analysis of NM and ion toxicity revealed that ions accounted for most of the toxicity detected in Ag and Cu NPs (>90% of the toxicity attributed to the ion fraction). However, for ZnO and CuO, this contribution amounted only to 25–30% and 6–30%, respectively. Thus, the toxicity of these substances was predominantly driven by the nanoparticles themselves.

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1.03.P-Tu012 Using Fathead Minnow Fish Embryo Tests to Parameterize Mechanistic Models for Environmental Risk Assessment Refinement

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New approach methodologies (NAMs) are increasingly used in environmental risk assessment to reduce reliance on live animal testing. Fish embryo toxicity tests (FET) show promise as proxies for acute live-animal fish tests and could be extended to early life stage (ELS) tests under the right assumptions. Mechanistic models serve as intermediates to bridge the acute-to-chronic gap but require suitable data to estimate relevant endpoints.

This study explores how growth and survival data from embryos exposed to a substance can be used to calibrate the toxicokinetic–toxicodynamic module of a Dynamic Energy Budget model. A modified FET protocol (OECD 236) was conducted on fathead minnow, extending test duration and incorporating destructive sampling for daily length measurements. Fathead minnow embryos are protected by a semi-permeable chorion. For most substances, permeability is unknown and estimates strongly influence extrapolation accuracy. The DEB model was calibrated on FET test endpoints and parameter values from the AddMyPet database, with TKTD parameters validated against an existing 33-day ELS study to estimate chorion permeability.

This approach could become an animal-free replacement for ELS endpoints with the right chorion permeability data and already shows utility combining growth data from the modified FET protocol with existing ELS observations to parameterize a mechanistic model for tier 2c refinement. This work demonstrates a novel approach to combine environmental risk assessment data and mechanistic models to expand refinement options in ecological risk assessments.

1.03.P-Tu013 2-Ethylhexanol induces cardiotoxicity and molecular neurobehavioral impairment in *Daphnia magna*

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The abundance of 2-ethylhexanol, which is a common plasticizer and solvent component, in aquatic ecosystems poses potential ecological risks. This study investigated the sublethal effects of 2-ethylhexanol on *Daphnia magna* by assessing physiological, biochemical, and molecular markers related to heart function and nervous system behavior. *D. magna* were treated with 1.11, 3.33, 10, and 30 mg/L of 2-ethylhexanol for 48 h. The endpoints assessed were cardiac and thoracic limb function; swimming behavior; neurochemical marker levels, such as acetylcholinesterase (AChE) and dopamine (DA); oxidative stress enzyme activity, including catalase (CAT), superoxide dismutase (SOD), and total nitric oxide (NO); and gene transcription associated with cardio- and neurotoxicity. Dose-dependent inhibition of cardiac rhythm and thoracic limb activity was also evaluated. Dysregulation of the transcription of the genes associated with cardiomyopathy, *sgcd*, *tmp3*, and *adcy5_1*, was observed. *D. magna* exhibited neurobehavioral disruptions, characterized by a reduction in body contact time and an increase in turn angle, supported by decreased dopamine levels and suppressed acetylcholinesterase activity. The oxidative stress response involved increased levels of CAT, NO, and SOD, with SOD demonstrating consistent upregulation at all concentrations. Significant changes in neurotoxicity-related genes (*drd2*, *slc6a3*, and *slc6a6*) highlighted the mechanistic connections to neurotransmitter dysregulation. These physiological, biochemical, and transcriptional alterations demonstrated that 2-ethylhexanol caused cardiotoxicity and neurobehavioral impairment in *D. magna* owing to oxidative stress. These ecological risks may disrupt predator-prey dynamics in aquatic ecosystems. Thus, reassessing the environmental safety thresholds of chemicals and identifying *Daphnia magna* as a sensitive model is essential for assessing sub-organismal toxicological effects.

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1.03.P-Tu014 Can Environmental RNA be Used to Assess Stress in Macro-organisms in the Field?

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Environmental nucleic acids (eNA), including both environmental DNA (eDNA) and environmental RNA (eRNA), have emerged as powerful tools for ecological monitoring. Unlike DNA, RNA is synthesized only for active genes in response to environmental changes and developmental processes. This characteristic makes the analysis of functional eRNA genes uniquely suited to providing physiological insights, such as information on reproduction, development, and stress responses, which cannot be inferred through eDNA analysis alone. However, the majority of studies focusing on functional gene detection remain confined to laboratory settings with a limited range of taxa.

In this study, I re-analyzed two published metatranscriptome studies on microbes that applied shotgun RNA sequencing to non-laboratory conditions, aiming to demonstrate that functional genes can be detected in environmental samples as eRNA released from macro-organisms. The re-analysis based on data from an aquaculture facility and an aquarium revealed that functional genes, such as *tlr4*, *lrp12*, and *elov11*, were detected as eRNA from Pacific oyster, whale shark, zebra shark, and green sea turtle. This result highlights the feasibility of using eRNA to assess biological processes, including immune responses and fatty acid metabolism. Despite these successes, challenges such as the dominance of microbial RNA, low sequencing depth, and RNA degradation were identified. This study provides a foundational direction for developing eRNA analysis as a non-invasive tool for ecological monitoring and the management of aquatic organisms in non-laboratory environments.

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1.03.P-Tu015 Toxicity of biohybrid and fossil fuel fumes to *Drosophila melanogaster*

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To support the transition away from fossil fuels, bio-hybrid fuels are being developed as potential replacements for conventional gasoline and diesel. One promising candidate is a ketone–ester–alcohol–alkane (KEAA) blend composed of ethanol, methanol, pentane, 3-methylbutanone, ethyl acetate and methyl acetate. In addition to engine performance, reduced toxicity is a critical consideration for the deployment of new fuels, particularly because human and environmental exposure to fuel vapors might occur during refueling, production, and

transport. Currently, standardized inhalation toxicity studies use vertebrate species (rats) to assess toxicity of volatile or gaseous pollutants. However, in order to minimize animal experimentation (3R principle), alternatives using invertebrate species need to be developed. Insects are considered especially sensitive to airborne contaminants due to their extensive tracheal respiratory system. Therefore, we established an acute inhalation toxicity assay using the fruit fly *Drosophila melanogaster*.

Adult *D. melanogaster* (strain OregonR) were exposed to concentration-series of KEAA and fossil-fuel vapors (gasoline E5). Water and acetone were used as negative and positive control, respectively. Mortality could be observed within one hour with LC50 values of 12.3 g/m³ and 23.8 g/m³ air for KEAA and gasoline (preliminary data), respectively.

Flies proved to be more sensitive to both pollutants than human lung cells (A549) in an air-liquid interface test setup, and reacted equally fast. The new biotest is versatile, easy to maintain and can be easily adapted to different insect species.

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1.03.P-Tu016 Potentials of Metabolomics-Based Early Indication of Drug-Induced Liver Injury (DILI) using In vitro and In vivo Methods

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Hepatotoxicity persists as a leading cause of post-market drug withdrawal, undermining the success of pharmaceutical development despite rigorous verification processes. Drug-induced hepatotoxicity, also known as drug-induced liver injury (DILI) is assessed through in vitro and in vivo screening in preclinical phase. Currently, the evaluation of chronic toxicity remains heavily dependent on in vivo models. Given the limitations associated with animal testing—including interspecies differences, extensive time requirements, and ethical issues—there is a growing demand for alternative testing methods. In vitro assays also take a relatively long period and harvests exposed cells that limit observation of time- and physiology-dependent cellular and molecular responses. Therefore, in this study, we applied extracellular metabolomics, a non-destructive methodology, to a traditional cytotoxicity assay. Our hypothesis was that changes in abundance of liver cell-derived biomolecules will be correlated with the magnitude of subsequent hepatotoxicity. We additionally expected that whether a drug is DILI or not could be predictable from the metabolomic profile patterns. HepaRG cell line was used for this experiment. Five DILI and non-DILI pharmaceutical substances were respectively selected, and exposure tests were conducted at concentrations showing high and low degree of hepatotoxicity. The exposed cells and the cell culture media were sampled 5 days later, pretreated, and analyzed using LC-QTOF-MS. Additionally, zebrafish embryo exposure testing was conducted to assess the correlation between in vitro metabolic signatures and in vivo toxicity at 72 hours post-fertilization (hpf). For this assessment, each compound was tested at its respective LC50 concentration. As results, we identified biomolecular differences in abundance for both intra- and extra-cellular, between the control and exposure groups. Furthermore, potential intracellular biomarkers for hepatotoxicity were identified through analysis of major metabolic pathways of biomolecules exhibiting significant differences between the groups. We also observed consistency between in vitro and in vivo results. These findings contribute to the basis for discovering potential extracellular biomarkers that can indicate DILI and non-DILI hepatotoxicity. At the same time, the study reveals the promises and limitations that require further studies.

1.03.P-Tu017 Fish Cell Lines in Pesticide Risk Assessment: Acute Toxicity and Bioaccumulation

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Environmental legislation requires rigorous chemical risk assessment before market authorization to safeguard aquatic ecosystems. Traditional approaches rely on toxicity tests that evaluate impacts on survival and bioaccumulation, yet these methods are resource-intensive and depend heavily on the use of live fish. Emerging alternatives, particularly fish cell line systems, offer to reduce and replace animal testing while being amenable to delivering mechanistically relevant data. The RTgill-W1 cell line, derived from rainbow trout gills, has gained international regulatory relevance through its adoption as OECD Test Guideline 249 for determining acute fish toxicity and can additionally be applied to investigate toxicokinetic processes, such as bioaccumulation.

In this study, we assessed the acute toxicity and bioaccumulation of three organic pesticides —trifloxystrobin, tebuconazole, and tembotrione— using RTgill-W1 cells. In vitro EC50 values aligned well with corresponding in vivo LC50 values for acute toxicity, with differences of less than one order of magnitude. Bioaccumulation

was quantified using kinetic depletion and steady state models and compared with in vivo estimates. Taking trifloxystrobin as an example, chemical analysis shows significant elimination in cell-containing exposures compared with controls, and biotransformation products could be identified, demonstrating the ability of the cell line to biotransform this pesticide. These findings indicate that fish cell line assays can capture both acute toxicity and bioaccumulation processes relevant to regulatory assessment. Future work will expand these evaluations to environmentally relevant temperatures and to gill cell lines from multiple fish species to investigate interspecific sensitivity differences.

1.03.P-Tu018 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, determination of appropriate concentration ranges based on 96-hour LC10 values and evaluation of the workflow and protocols regarding technical feasibility and practicability in exemplary trials.

1.03.P-Tu019 Filling in the gaps to support the application of alternative methods (OECD TG 236 and 249) to replace acute fish toxicity testing

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Information on acute fish toxicity is a data requirement under several EU legislations dealing with environmental safety of chemicals around the world. Historically, standardised in vivo testing has been used to fulfil such data needs, for example using the OECD test guideline (TG) 203. Ethical and practical reasons are promoting alternative methods, such as tests on fish embryos (OECD TG 236) or a fish gill cell line (OECD TG 249), adopted in 2013 and 2021, respectively. Replacement of the in vivo acute fish test for regulatory purposes requires a systematic comparison with the alternatives as far as data availability across methods allows, especially to identify areas of chemical space where over- or under-prediction of toxicity may occur.

The European Chemicals Agency (ECHA), with the support of a consortium composed of aQuaTox-Solutions, INERIS and Ricardo, ran a project to gather OECD TG-compliant data for the in vivo and the two alternative assays to analyse their concordance and to further advance knowledge on the applicability domain of the OECD TGs 236/249. Specific focus was on the areas of chemical space where these methods offer an equal or higher level of protection to fish and where uncertainties on the applicability still remain due to technical limitations or biological implications. Data on tests performed according to OECD TG 203 and stored in the International Uniform Chemical Information Database (IUCLID) were curated for comparison as a reference. Data generated using OECD TGs 236 and 249 were collected through a literature search from peer-reviewed journals, reports from research projects and publicly available databases. Information was harmonised and recorded using a tailor-made template.

Statistical analyses were performed on substances with data available from studies covering all three considered TGs. These analyses included regressions comparing effect levels between assays and enrichment analyses to

identify effect level ratios that deviated significantly from one. The substances were partitioned according to their physicochemical properties and their expected mode of action to determine for which substances and under which conditions one test may be more conservative than another. We will present the types and characteristics of substances for which the applicability of the alternative assays has been demonstrated, as well as those for which it has not yet been demonstrated and for which additional information is needed.

1.03.P-Tu020 Deriving Effect-Based Trigger Values in Human Serum Using Non-Target Suspect Screening Data, Reference Doses, and Toxicokinetic Modelling

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We encounter a multitude of chemicals in daily life. Thus, it is vital to understand the internal exposure to chemical mixtures in human biomonitoring to better assess the impact of chemical pollution on health. Effect-based bioassays provide integrated biological readouts that captures the cumulative effects of all chemicals with similar sites of action present in a sample, making them powerful tools for mixture exposure assessment. Yet, the high sensitivity of these high-throughput assays can lead to overestimation of the effects of chemical mixtures on human health because also endogenous chemicals may trigger effects in these assays.

To differentiate between acceptable and unacceptable bioassay responses to the mixture of contaminants found in human samples, we need effect-based trigger values (EBTs) for bioassay responses. Establishing such EBTs remains challenging due to the complexity of human exposure profiles, the diversity of chemical signatures in serum, and the lack of comprehensive quantitative datasets that reliably link observed bioactivity to internal concentrations (e.g. blood levels) and health-based reference values. This limitation highlights the need for innovative strategies to derive scientifically robust benchmarks.

Our novel strategy for deriving EBTs for human serum combines high-resolution mass spectrometry-based non-target and suspect screening data of pooled serum extracts, with established reference doses from regulatory bodies (e.g., EFSA, ECHA), and toxicokinetic modelling. This workflow estimates internal concentrations corresponding to health-protective external exposure limits. By aligning bioassay responses with these modelled internal thresholds, we establish EBTs that reflect realistic exposure scenarios and regulatory guidance rather than arbitrary effect levels. This integration accounts for mixture effects, uses non-quantitative analytical data, and prioritizes chemicals driving observed bioactivity enhancing interpretability for risk assessment.

The application of this workflow to human serum samples demonstrates that EBTs derived through combined chemical-biological modelling can provide accurate and context-specific benchmarks compared to conventional effect-based approaches. This strategy offers a pathway for harmonizing state-of-the-art chemical occurrence data, bioassay outcomes, and regulatory standards in human biomonitoring programs, supporting improved health protection and evidence-based decision-making.

Disclaimer/Disclosure:

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1.03.P-Tu021 Biological Effects of Microcapsules 3

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Microcapsules are small particles that encapsulate chemicals in an inner core. Similar in size to microplastics, they enable controlled and sustained release. The environmental fate of the encapsulated chemicals depends on the capsule matrix, which can slow degradation, enhance stability, or increase mobility, thereby altering hazard profiles.

On the other hand, these properties can be advantageous in aquatic ecotoxicity testing. Microencapsulation stabilizes degradable chemicals and reduces volatility, allowing evaluation of substances otherwise difficult to test with standard waterborne methods. It also shifts exposure in aquatic organisms from dermal or branchial absorption to oral intake, offering new insights into toxicokinetics and toxicity.

To address these challenges, we propose a Chemical Delivery System (CDS) that delivers chemicals to fish via microcapsules (MCs), particularly those unsuitable for waterborne administration. This approach creates

exposure conditions more comparable to mammalian studies.

In this presentation, we report our investigation of the chemical load per microcapsule and present the results of toxicity assays conducted using this microcapsule-based exposure system.

Disclaimer/Disclosure:

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1.03.P-Tu022 Bioaccumulation Assessment Using New Approach Methodologies for Difficult Substances

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Screening for bioaccumulation traditionally relies on surrogate metrics such as octanol-water partition coefficient (log Kow), which has been widely used and accepted for decades and underpins many quantitative structure-activity relationships ((Q)SARs) models.

However, the accuracy of the log Kow for the bioaccumulation assessment is limited to certain substance classes and primarily reflect one accumulation mechanism – partitioning into storage lipids. For substances such as surfactants, ionisable compounds, or per- and polyfluoroalkyl substances (PFAS), bioaccumulation may involve alternative mechanisms, including specific biological binding interactions and depuration processes.

To address these limitations and improve bioaccumulation assessment for a broader range of substance, including difficult test substances, we have developed a non-animal bioaccumulation testing strategy combining up to five in chemico / in vitro assays, followed by in silico predictions. The tests include:

- 1) Log Kow - to account the partitioning to storage lipids;
- 2) Octanol/air partition coefficient (Koa) - to account for air-breathing organisms;
- 3) Membrane liquid/water partition coefficient (Kmw) - to avoid the estimation of lipid interactions using Kow;
- 4) Bovine serum albumin (BSA) solution/water partition coefficient (Kbsa) - to account for protein binding accumulation processes;
- 5) Determination of in vitro intrinsic clearance in rainbow trout liver cells (OECD 319A RT-HEP) - to account for biotransformation processes.

Each test aids in the removal of specific uncertainties related to substances that may accumulate via other processes than that indicated by log Kow. These data can inform weight-of-evidence approaches and/or serve as input for the BAT “Bioaccumulation Assessment Tool” QSAR model, improving accuracy of intermediate property predictions (e.g. biotransformation) / BCF predictions compared to Kow-based estimates alone. Incorporating these parameters into modelling software increases the precision, accuracy and predictability in an in vitro - in vivo extrapolation (IVIVE) approach.

The testing strategy proposed permits robust conclusion on bioaccumulation under PBT (Persistent, Bioaccumulative, Toxic) assessment regulatory frameworks. Here we present a case study and outline in which specific instances these tests are of value.

1.03.P-Tu023 Establishing Hemocyte Cell Cultures from Parhyale hawaiiensis: A Key Step Toward New Approach Methodologies (NAMs) in Marine Ecotoxicology

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Toxicology and ecotoxicology rely on model organisms to assess chemical and environmental hazards. However, the traditional use of vertebrates raises ethical concerns, encouraging the development of New Approach Methodologies (NAMs), such as in vitro cell culture systems. Despite advances with insect cell lines, marine invertebrate cell cultures remain scarce. The amphipod *Parhyale hawaiiensis* is a promising model organism, yet no established cell culture system exists for this species.

This study aimed to develop primary hemocyte cultures of *P. hawaiiensis* to support NAM-based assays. Adults were maintained under controlled salinity, temperature, and feeding conditions at the Laboratory of Ecotoxicology and Genotoxicity (LAEG) of the Universidade Estadual de Campinas (UNICAMP). Hemolymph was extracted using glass capillaries and cultured in Leibovitz's L-15 medium supplemented with FBS, antibiotics and Red Sea Salt to adjust salinity. Cultures were maintained in 96-well plates at 26 °C and

monitored daily.

Establishing this culture presented significant challenges due to the organism's very small size, which limits the volume of hemolymph obtained and makes it difficult to maintain sufficient cell numbers. Even so, results demonstrated the successful formation of primary cultures: cells adhered to the plate surface within 4 hours and remained viable for up to two weeks with medium replacement. This achievement represents an important milestone for the application of NAMs in marine ecotoxicology and provides the foundation for developing a continuous cell system for this amphipod.

We are currently optimizing the visualization of adhered cells using confocal microscopy, aiming at fluorophore staining and future structural characterization. As next steps, we plan to compare adverse effects on cultured hemocytes and also on those derived from exposed animals using different contaminants to strengthen the use of these cultures as a robust tool within NAMs.

The authors acknowledge the financial support from FAPESP (Grant 23/10693-5), which was fundamental for this research.

1.03.P-Tu024 Checklist-based quality criteria for generating and reporting acute fish cell line data in the FACT database.

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The RTgill-W1 fish cell line assay, a bioassay that is validated and standardized as the OECD Test Guideline (TG) 249, has shown great promise as an alternative method to fish acute toxicity testing (e.g., OECD TG 203). Despite its rigorous validation and adoption as ISO standard 21115 and OECD TG 249, its uptake is facing barriers such as reluctance in regulatory acceptance, often due to a lack of understanding and confidence in using the data, and some data not being publicly available. This could be helped by a comprehensive characterization of the assay's applicability domain, i.e., the chemical space in which the assay can be expected to perform reliably, which, however, requires an overview of all available data.

To collect all available fish cell acute toxicity data, we created the Fish Acute Cell Toxicity (FACT) database as a continuously updated resource. Currently, the FACTdb contains toxicity (cell viability) data on over 100 chemicals represented through EC50, EC10, and non-toxic concentrations (NtC) values, calculated using a consistent analysis workflow. In the process of data curation, it became evident that even with such a standardized assay, there is still variation in how the assay is conducted and the data is reported. This often leaves questions regarding the validity and suitability of the data for inclusion into the database. Accordingly, we created checklist-based quality criteria that sensitize users towards key elements of data generation and reporting. This checklist follows the OECD guidance document 147 requirements "on the Generation, Reporting and Use of Research Data for Regulatory Assessments". It therefore requires information across the categories of chemical properties, experimental setup, and validity criteria. The checklist serves as a quality assessment before data are entered into the FACT database. It assigns guideline adherence levels from 1 to 3: Level 1 indicates full compliance with OECD TG 249, Level 2 reflects adherence with some deviations (e.g., alternative plate formats), and Level 3 concerns relevant research data (e.g., other fish cell lines).

We aspire for the combination of the checklist and FACT database to strengthen both the confidence of the regulatory community in how to best conduct the assay, improve the reporting quality of generated data, motivate further uptake of the assay, and help towards higher regulatory acceptance by further characterization of the evolving applicability domain.

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1.03.P-Tu025 Catching the Risk: Leveraging New Approach Methodologies to Address Acute Fish Toxicity in Plant Protection Product Assessments

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In the European Union (EU), the authorization of plant protection products (PPPs) and their active substances involves a comprehensive evaluation of environmental risks to various taxonomic groups. One of the

requirements is assessing the acute toxicity towards fish (Commission Regulations (EU) 283/2013 and 284/2013). Concurrently, there are ongoing initiatives aimed at reducing or completely eliminating vertebrate testing within the EU and other regions. Over recent decades several New Approach Methodologies (NAMs) have been developed to predict the acute toxicity towards fish such as the fish embryo toxicity assay (FET, OECD TG 236) and the rainbow trout gill cell line assay (RTgill W1, OECD TG 249). However, these alternative methods have yet to be integrated into the registration process for PPPs as is already done or under development within other legislations (e.g., REACH, cosmetics, German wastewater treatment assessment). In a recent publication, we found that the risk assessment for less than 10 % of the active substances registered in the EU in 2022 was primarily based on the acute risk towards fish in an aquatic first-tier assessment (Adams et al., 2025). In a subsequent step we assessed the modes of action (MoAs) of these substances and identified various MoAs that potentially cause a higher acute toxicity towards fish. Our research focuses primarily on the substance classes that have shown an increased acute risk to fish, rather than on those classes that have demonstrated no or only a low acute risk for fish compared to other aquatic endpoints. Ultimately, the goal of this research is to develop a tiered approach to cover fish acute toxicity starting with NAMs and only conducting vertebrate tests as a last resort to ensure ecological safety. By focusing on critical substance classes, we aim to better understand the utility of NAMs in ecotoxicological risk assessments as these might not be covered by NAMs. Preliminary results from FET assays with *Danio rerio* demonstrate a significant overlap in effect concentration ranges with results from acute fish tests. Further findings will be presented at the conference, contributing to the ongoing discussion on the integration of NAMs into the legislation framework of PPPs risk assessment.

1.03.P-Tu026 Machine Learning-Enhanced High-Content Screening Platform for Genotoxicity Assessment in Human HepaRG Cells

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The assessment of chemical-induced genotoxicity remains a critical challenge in safety evaluation, particularly given the limitations of conventional methods, which often lack metabolic relevance and multimodal integration. To address this, we developed a high-content screening (HCS) platform combining immortalized human HepaRG cells—capable of intrinsic metabolic activation—with machine learning (ML) for mechanism-based genotoxicity classification.

HepaRG cells were cultured in 96-well plates and exposed to 24 mode-of-action (MOA)-characterized genotoxic agents (16 clastogens, 8 aneugens) across nine concentrations. A triplex fluorescent staining protocol (Hoechst 33342, ethidium bromide/acridine orange) enabled high-content imaging of nuclear morphology, apoptosis, and membrane integrity. From >1,000 fields per well, 51 phenotypic parameters were extracted, including micronucleus (MN) counts and morphometric features. A three-tier feature selection process narrowed these to 13 discriminative parameters for ML modeling.

Three algorithms—Random Forest (RF), Support Vector Machine (SVM), and Gradient Boosted Decision Trees (GBDT)—were benchmarked. SVM achieved the best performance with 75.5% test accuracy and an AUC-ROC of 0.895, effectively distinguishing clastogenic from aneugenic mechanisms. The optimized pipeline was applied to predict MOA for 27 plastic additives with unknown genotoxicity profiles, yielding consistent multi-model predictions for 89% of compounds.

This integrated approach offers a physiologically relevant, high-throughput, and interpretable platform for mechanism-based genotoxicity assessment, overcoming key limitations of existing methods.

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1.03.P-Tu027 Zinc- and Copper-Containing Metal Fumes Inhibit Inflammatory Responses in THP-1-Derived Human Macrophages

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Occupational exposure to metal fumes increases the risk of severe pneumonia among metal workers, though the immunological mechanisms remain unclear. This study investigated whether zinc oxide (ZnO) and copper oxide (CuO) particles suppress human macrophage inflammatory responses to bacterial pathogens. Human THP-1-derived macrophages were differentiated by exposure to phorbol 12-myristate 13-acetate for 48 h and subsequently exposed in vitro to bacterial pathogen-associated molecular patterns (PAMPs): lipopolysaccharide (LPS; 2 or 20 ng/mL), lipoteichoic acid (LTA; 100 or 1000 ng/mL), or peptidoglycan (PGN; 1000 or 3162 ng/mL), with or without 2 µg/mL CuO/ZnO particles generated by metal inert gas soldering. Particles were nanosized (40 to 250 nm) with a predominantly spherical morphology as observed by scanning electron microscopy. The elemental composition primarily consisted of 49.42% Cu and 47.27% Zn, as measured by inductively coupled plasma mass spectrometry. Energy-dispersive X-ray spectroscopy confirmed the elemental composition, and oxidation states were inferred from oxygen detection and the known properties of CuO/ZnO. TNF and IL-6 mRNA expression was quantified by qPCR 4 h post-exposure (n=5), and secreted protein levels were assessed by electrochemiluminescent immunoassay after 24 h (n=3). PAMP stimulation induced a dose-dependent increase in TNF and IL-6 mRNA and protein, which was significantly inhibited by 2 µg/mL CuO/ZnO particles. Inhibition was more evident with TNF than with IL-6. Particle exposure with CuO/ZnO only significantly suppressed TNF protein secretion, while IL-6 was unaffected. Cell viability remained high (97.5%) at 2 µg/mL, with an IC50 for cytotoxicity of 6.7 µg/mL. These findings indicate that CuO/ZnO particles can inhibit key macrophage immune responses to bacterial stimuli, providing a potential mechanistic explanation for the increased risk of severe pneumonia among metal workers, supporting current pneumococcal vaccination recommendations for occupationally exposed individuals, and highlighting the value of exposure limits and preventive measures.

1.03.P-Tu028 Can gene expression be used to detect endocrine activity in zebrafish embryos?

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Endocrine disrupting compounds (EDCs) are substances that can interfere with the normal functioning of the hormonal systems, posing a potential risk to both wildlife and human health. To be classified as EDCs, compounds must exhibit endocrine activity at the relevant receptor or enzyme and as a consequence, cause population-relevant adverse effects in either the exposed organism or its offspring. Current regulatory frameworks make use of a range of in vivo assays in adult vertebrate species to assess both aspects of endocrine disruption. However, the ethical concerns associated with animal suffering have generated increasing cross-stakeholder momentum to align regulatory approaches with the 3Rs principles (Refinement, Reduction, and Replacement of animal experiments) and to reduce dependence on animal-based test systems. According to European Directive 2010/63/EU regarding the protection of animals used for scientific purposes, zebrafish embryos model are not considered as a protected life stage up to 120 hours-post-fertilization (hpf), as the embryos are not capable of independent feeding at this stage. This research investigates the potential of fish embryos as a non-animals alternative model for assessing the endocrine activity of chemicals across three EAS modalities. The focus of this study is to determine whether the key components of the sex endocrine system in zebrafish are expressed and functional during the early developmental period up to 96 hpf, which is essential for generating a reliable transcriptional response associated with endocrine activity.

1.03.P-Tu029 Predicting Pulp and Paper Mill Effluent Toxicity Using the RTgill-W1 Assay

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Around the world, regulatory programs are demanding a shift from traditional vertebrate-based toxicity test methods to alternative approaches. In Canada, this was introduced in legislation aiming to phase out whole animal vertebrate toxicity testing by 2035. As these tests use millions of vertebrates to inform several regulatory programs, the development of replacements is desperately needed. One of the most extensively used regulatory tests exposes rainbow trout (*Oncorhynchus mykiss*) to industrial and municipal effluents to determine their compliance with national and provincial legislation. Our research explores an alternative approach, based on the OECD 249 and ISO 21115 methods, that uses cell viability indicators in a rainbow trout gill cell line (RTgill-

W1) to assess the acute toxicity of effluent samples. Using pulp and paper mill effluents from 5 unique sources, we assessed the robustness of RTgill-W1 assay to detect effluent toxicity through reference toxicant testing in non-toxic effluents (n = 42) and control media (n = 54) backgrounds. The sensitivities of both test methods were assessed using effect-causing effluent samples (n = 20). In all of these effect-causing effluents, the RTgill-W1 assay performed similarly to the rainbow trout test and proved generally more sensitive to pulp and paper mill effluent. We also determined the use of this assay as a tool to identify common toxicants of pulp and paper mill effluents to demonstrate its potential for investigative testing. This research provides critical information about the sensitivity of a potential replacement to the most widely used test in effluent regulations globally.

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1.03.P-Tu030 Long-term In Vitro Exposure Strategy to Study Micro- and Nanoplastic Carcinogenicity

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Human populations are increasingly exposed to many environmental pollutants over long periods, which may lead to carcinogenic impacts. Traditional animal assays cannot support the large testing needs for such agents, which highlights the need for human-relevant in vitro approaches based on new approach methodologies. This work addresses this challenge by applying a long-term in vitro exposure strategy combined with multiple transformation biomarkers to evaluate chronic effects of airborne micro- and nanoplastics, a widespread pollutant with growing concern for human health.

We exposed human bronchial epithelial cells continuously for 30 weeks to PET particles representative of real-life exposure scenarios. The micro- and nanoplastics size, morphology and stability were characterised, and cell uptake was confirmed. Our exposure strategy enables time-resolved assessments; therefore, key biomarkers of transformation were examined at mid and late stages, including DNA damage, anchorage-independent growth, and invasion capacity. Transcriptomic profiling at three time points provided mechanistic insight into progressive cellular changes.

Cells showed no early effects, but after 30 weeks clear transformation features emerged. Genotoxicity appeared only at the latest stage, suggesting it may be a consequence rather than an initiating event. Growth in soft agar and invasive behaviour also increased only after long-term exposure, indicating a progressive acquisition of malignant traits. Gene expression analyses confirmed these trends: the number of altered genes rose over time, many linked to cancer pathways. Notably, genes associated with epithelial–mesenchymal transition and lung cancer were strongly upregulated, supporting the phenotypic evidence and pointing to mechanisms related to oncogenic activation.

Overall, this study demonstrates that chronic exposure to PET particles can induce progressive transformation in human lung cells. The approach reproduces realistic long-term conditions and uncovers effects that short-term assays would miss. These findings strengthen the case for considering chronic low-dose exposure to micro- and nanoplastics in risk assessment and illustrate how long-term in vitro models can advance carcinogenicity testing and mechanism-based evaluation of emerging pollutants.

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1.03.P-Tu031 Development of Biocide-Specific Quantitative Structure-Activity Relationship(QSAR) model to Predict Developmental Toxicity to Zebrafish Embryos

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Objective: The objective of this study was to develop a Quantitative Structure–Activity Relationship (QSAR) model to predict the acute toxicity of biocides to zebrafish embryos.

Methods: We produced developmental toxicity of 74 biocides by using zebrafish embryo. Among them, except for inorganics and mixture compounds the calculated median lethal concentration (LC50) values of 42 biocides were used as the dataset for QSAR model development. Chemical space analysis using a Tanimoto heat map, LC50 distribution, and Structure-Similarity Activity Trailing (SimilACTrail) confirmed that the dataset was suitable for fingerprints and descriptors were calculated as input features. The molecular features were split into training and test sets at an 8:2 ratio using the Kennard–Stone algorithm, and a genetic algorithm was applied to identify optimal feature combinations for predicting LC50 values. Finally, a Multiple Linear Regression (MLR) model was developed, and its predictive performance was evaluated using R^2 , root mean square error (RMSE), and 5-fold cross-validation (CV).

Results: Toxicity testing showed that 73 individual biocides (98%) caused embryonic mortality with a median lethal concentration ranging from 0.73 to 58.82mM. The diversity of chemical structures and LC50 distribution confirmed that the dataset was appropriate for model development. The SimilACTrail map showed that toxicity is largely structure-driven, with one chemical pair highlighting chlorine as a key determinant. Toxicity testing showed that 73 individual biocides (98%) caused embryonic mortality with a median lethal concentration ranging from 0.73 to 58.82mM. The diversity of chemical structures and LC50 distribution confirmed that the dataset was appropriate for model development. The SimilACTrail map showed that toxicity is largely structure-driven, with one chemical pair highlighting chlorine as a key determinant.

Conclusion: The developed fingerprint-based QSAR model demonstrated strong predictive performance, indicating its suitability for assessing biocide toxicity. Both the fingerprint-based interpretation and the SimilACTrail analysis consistently showed that biocides containing halogen atoms exhibit greater developmental toxicity in zebrafish embryos. This study demonstrates a solid and reliable QSAR framework for biocide toxicity evaluation with clear applicability for regulatory assessment and chemical screening.

1.03.P-Tu032 Defining the Domain of Applicability: A Case Study to Increase Confidence in Toxicogenomic Tools for Ecological Risk Assessment

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Toxicogenomics offers a powerful means to characterize the molecular effects of environmental chemicals, often with reduced animal testing. Its use in human health assessment has grown rapidly in the last decade. In contrast, applications in ecological risk assessment remain limited, and uncertainty remains regarding how toxicogenomic responses translate to ecologically relevant outcomes. The objective of this research was to increase confidence in, and advance the application of, toxicogenomic approaches within ecological risk assessment.

A landscape review was conducted to evaluate current toxicogenomic applications in ecological assessments and to identify existing regulatory considerations. The primary focus of research among identified applications was the development and evaluation of ecological transcriptomic points of departure (tPODs). Although regulatory agencies are beginning to consider toxicogenomic data within weight-of-evidence evaluations, no formal ecological risk assessment frameworks currently incorporate these data. The review identified three key research gaps: (1) alignment between qPCR-based and whole-transcriptome tPODs; (2) ability of transcriptomic approaches to resolve species-sensitivity differences; and (3) concordance of in vitro and in vivo transcriptomic responses.

To address these gaps, a case study was designed using unpublished RNA sequencing and EcoToxChip qPCR datasets from fathead minnow and rainbow trout exposed to ethinylestradiol during early life stages. Comparative analyses will evaluate sensitivity, reproducibility, and variability across technologies, species, and exposure durations, and assess concordance with established apical effects and reference values. EcoToxChip data generated by a contract research organization will also assess feasibility outside academic and governmental settings.

The case study will address the performance and limitations of transcriptomic new approach methods (NAMs),

inform best practices for their use, and support their broader application in ecological assessments. Collectively, this work strengthens the evidence base needed to incorporate toxicogenomics into ecological risk assessment, and identifies priority areas for future research, including cross-trophic evaluations and applications in chemical grouping and read-across.

1.03.P-Tu033 Are Spheroids or Organoids the Real Game-Changers for Micro(nano)plastics Health Impact Studies?

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Plastics have emerged as one of the most impactful alterations on the planet. Since their introduction in the 1950s, production has increased, reaching 430.9 million tonnes in 2024. Poor end-of-life management and the widespread use of single-use plastics have contributed to their accumulation in the environment, where they degrade into micro(nano)plastics (MNPs). These smaller particles have high bioavailability, with numerous studies, mainly with aquatic organisms, reporting adverse effects on reproduction, immunity, metabolism, and behaviour. Such findings raise concern about possible impacts on humans, who may be exposed through ingestion of contaminated food and beverages, inhalation of airborne particles, and dermal contact. Recent studies have proven that humans are incorporating (e.g., detection in blood) and releasing (faeces) these particles. However, current knowledge of their effects on humans remains limited and is largely based on rodent studies or 2D in vitro cultures, both of which present uncertainties due to species-specific physiology or the simple nature of monolayer systems.

Advanced 3D human cell culture models may help overcome these limitations. Spheroids and organoids, for example, are multicellular structures that better reproduce tissue architecture and cellular interactions, offering more physiologically relevant platforms without the ethical constraints of animal or human experimentation. Their use is particularly valuable given the diversity of MNP characteristics (e.g., polymer type, size, shape, surface charge, age, and chemical additives) that can influence their toxicity.

This study aims to integrate and increase current knowledge on the relevance of 3D systems for MNP research, highlighting their advantages and limitations with a focus on human models. Two approaches were used. First, a literature review of studies investigating MNP effects on human health. Second, in vitro experiments using human cell lines (e.g., HEK293T) exposed to fossil-based polymers (e.g., polymethylmethacrylate) and bio-based polymers (e.g., polylactic acid) in both 2D and 3D cultures (spheroids). Overall, data show that 3D systems provide enhanced biological relevance and improved predictive power for human risk assessment of micro(nano)plastics.

1.03.P-Tu034 Predicting Acute Fish Toxicity with the RTgill-W1 Cell Line Assay: New Insights and Regulatory Applications using Fragrance Chemicals as Case Studies

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The RTgill-W1 cell line assay was recently adopted as an OECD Test Guideline (TG) 249 and several studies have been published demonstrating its usefulness to predict acute toxicity to fish as a non-animal alternative to the conventional approach (OECD TG 203). Here we present new insights on the accurate prediction of in vivo LC50 values, and regulatory applications using fragrance chemicals as case studies.

According to TG 249, the predicted LC50 is equal to the lowest EC50 among three cell viability endpoints based on geometric mean measured concentrations. As an alternative, regression equations based on 38 fragrance chemicals translating the EC50 (metabolic activity endpoint) and measured concentrations (arithmetic mean, geometric mean) to the LC50 was developed by plotting EC50 against the corresponding in vivo LC50 values. In this study, the two approaches were applied for the fragrance chemicals used previously and for 11 additional chemicals as test set and compared to in vivo LC50 data. Both approaches can predict LC50 values accurately, yet the regression approach is more conservative. In addition, TG 249 was applied for the assessment of aquatic toxicity on 30 newly tested fragrance chemicals and two cosmetic ingredients including chemicals tested for regulatory purposes. For animal welfare reasons, nine in vivo studies required for regulatory dossiers were performed using the threshold approach and were in line (within a factor of 3-times) with predicted LC50 using the TG 249. A good agreement of predicted LC50 by the RTgill-W1 cell line assay and QSARs was observed (n=5) and in vitro data were also applied for five read-across studies. For three poorly water-soluble (< 1 mg/L) fragrance ingredients, effects on the RTgill-W1 cell line were evaluated using water saturated solutions demonstrating no effects at saturation. In conclusion, the TG 249 is a valuable tool to predict LC50 values for hydrophobic (highest log Kow 6.6) and volatile fragrance ingredients including complex substances (multi-constituent, UVCB) and has been successfully applied for REACH registrations to avoid animal testing. This

presentation will clearly demonstrate that fragrance molecules of broadly varying physico-chemical characteristics and differing chemistries fit well within the domain of applicability of the OECD TG 249.

1.03.P-Tu035 Mechanism-Informed PFAS Toxicity Classification Using Integration of Transcriptomics and Computational Platforms

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PFAS comprise thousands of chemicals, necessitating a grouped management strategy; however, even classification approaches are limited due to scarce toxicological data. We aimed to evaluate the utility of multi-dimensional information from chemical structure, bioactivity, and transcriptomics for classifying the developmental toxicity of PFAS in zebrafish embryos. We further integrated them to enhance classification performance and identify potential biomarkers for mechanism-anchored classification. A total of 15 PFAS were classified by developmental toxicity in zebrafish embryos. Those Chemotype, ToxCast, and transcriptomics data were collected from the ToxPrint Chemotyper, CompTox Dashboard, and GSE252004 respectively. To evaluate classification performance of individual- and integrative data, five machine learning algorithms were employed, and unsupervised analyses were employed to validate classification performance. Individual platforms showed poor performance (maximum accuracy ? 0.53). Integration of 44 selected features achieved perfect classification with high generalization (permutation mean ? 0.93). Further optimization identified 12 features: one structural feature (bond:COH_alcohol_pri-alkyl), two ToxCast assays (keratin 8/18, deiodinase-1), and nine pathways including NOD-like receptor signaling, endocytosis, mitochondrial ATP synthesis, and proteostasis. These features showed revealed toxicity-specific mechanisms: high-toxicity PFAS disrupted immunity and energetics; moderate-toxicity perturbed endocytic pathways; low-toxicity triggered adaptive and receptor-mediated program. We demonstrated that multi-platform integration efficiently classified developmental toxicity of PFAS and provided the corresponding potential biomarkers. This new approach could be applied to regulatory prioritization of data-poor PFAS and their mechanism-anchored grouping.

1.03.P-Tu036 High-Throughput C. elegans Toxicity Screening as a 3R-Aligned Whole-Organism Platform for Chemical Prioritisation

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High-throughput screening (HTS) using *Caenorhabditis elegans* represents a promising 3R-aligned whole-organism approach for early-tier chemical evaluation. Combining short life cycles, low cost, and exceptional genetic tractability, *C. elegans* allows rapid assessment of developmental, behavioural, and mechanistic endpoints that are challenging to capture in cell-based assays and impractical in vertebrate tests. This review synthesises current HTS platforms—including microfluidics, behavioural phenotyping, large-particle flow-based sorting, and image-based developmental assays—and evaluates their suitability for screening, prioritisation, and integration into modern New Approach Methodologies (NAMs).

Image-based HTS platforms exhibit the strongest cross-species concordance, showing close alignment with apical toxicity endpoints in fish and aquatic invertebrates. Behavioural HTS systems complement these assays by revealing neuroactive and sublethal effects at low doses, leveraging conserved neurotransmitter pathways and characterised neurodevelopment. We also highlight considerable intraspecific variation across wild *C. elegans* strains, demonstrating the importance of genetic diversity for capturing realistic ranges of chemical susceptibility and enhancing the generalizability of HTS results.

We outline the key technical and biological considerations required for broader adoption of nematode HTS, including feeding regimens, media effects, evaporation and absorption artefacts, and the need for dose-setting strategies that reliably capture full concentration-response curves. Persistent gaps—particularly limited overlap between nematode HTS and mammalian datasets—underscore the need for coordinated testing and development of shared chemical reference panels. When combined with the AOP framework, these comparative datasets will help clarify when nematode responses reflect conserved mechanisms relevant across taxa.

By integrating whole-organism biology with scalability and low animal-use burden, *C. elegans* HTS offers a compelling 3R-supportive alternative for chemical prioritisation and screening. We provide recommendations

for harmonisation, validation, and future investment to establish nematode-based HTS as a robust component of next-generation risk assessment.

1.03.P-Tu037 Enhancing Chemical Design with New Approach Methodologies: Evaluating Predictive Ecotoxicology Models for Structurally Similar Chemicals

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The Safe and Sustainable by Design (SSbD) concept is a pre-market innovation approach aiming to minimise adverse human and environmental impacts from a chemical's inception in the design process. The integration of computational prediction models into design processes addresses the need to efficiently assess, compare and narrow down the number of candidate chemicals. A key challenge for the implementation of this is the need for reliable predictive models for chemical hazard indicators. This study evaluated the capabilities of existing in silico models for predicting the ecotoxicity of 17 selected structurally similar triazine compounds, including four known persistent, mobile, and toxic (PMT) chemicals. Acute toxicity data for *Daphnia magna* and *Danio rerio* (zebrafish) embryos were experimentally generated following OECD test guidelines 202 and 236. Quantitative Structure-Activity Relationship (QSAR) models from ECOSAR (US EPA EPISuite) and VEGA HUB modelling suites relevant to these endpoints were selected for analysis. Requiring only a chemical structure as input, QSAR models have high potential for efficient integration into chemical design processes. The performance of the models for structurally similar chemicals was evaluated by comparing the predicted effect concentrations with the experimental data, as well as ranking the toxicity of the chemicals based on experimental and in silico data. The study is currently being expanded to include additional computational models, such as machine learning approaches applying harmonised datasets. This will contribute to a broader, more comprehensive evaluation of available predictive models. Preliminary results from this study show variability in the predictive accuracy of the currently included models, indicating a need for their improvement before they can be effectively integrated into SSbD workflows. The conclusions of this study provide important guidance on which models can already be applied in chemical design contexts, contributing to the integration of new approach methodologies (NAMs) into early chemical design processes, supporting the development of safer chemicals.

1.03.P-Tu038 Advancing Fish-Specific Immunotoxicology through NAMs: Can Non-Immune Zebrafish Cell Lines Detect Drug-Induced Immunomodulation?

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Immunotoxicity is increasingly recognised as a regulatory priority in environmental risk assessment; however, assessing the effects of chemicals on the fish immune system remains heavily reliant on in vivo studies. Our previous in vivo work has shown that several immunomodulatory pharmaceuticals elicit comparable immune responses in fish and humans, demonstrating conserved mechanisms but also highlighting the need for scalable, animal-free approaches. Yet the absence of established fish immune cell lines restricts the development of higher-throughput immunotoxicology NAMs. Given the documented sensitivity of non-immune cell types to immune stimuli, we tested the hypothesis that the fibroblast-like zebrafish ZF4 line could detect drug-induced immunomodulatory activity and serve as a mechanistically informative platform for fish-specific NAMs.

Using TNF α as an anchor reference immunostimulant, we characterised early and late responses in ZF4 cells through IL-6 secretion assays, high-content cell-painting morphological profiling, and RNA-seq-based transcriptomic analysis. This data revealed rapid IL-6 release, distinct phenotypic fingerprints, non-monotonic morphological patterns, and time-resolved transcriptional changes, indicating that ZF4 cells can sense cytokine-mediated perturbations despite their non-immune origin. Building on this foundation, we expanded the approach to a broader panel of cytokines representing diverse inflammatory states, alongside a selected set of pharmaceuticals with known immunomodulatory activity in mammals.

In this presentation, we will discuss the ability of ZF4 cells to discriminate across cytokine- and drug-induced immune-relevant perturbations, and evaluate their potential application as a scalable, high-content NAM for fish immunotoxicology. By integrating cytokine benchmarks, drug-induced phenotypes, and multimodal readouts, this work provides a case study illustrating how non-immune fish cell lines could contribute to IATA-compatible assays and help reduce reliance on traditional in vivo immunotoxicity studies.

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1.04 Bridging Science and Policy: The Role of Adverse Outcome Pathways in Research and Regulatory Practices to Support Next Generation Risk Assessment

1.04.T-01 Neural crest cell impairment as a conserved developmental target of retinoid signalling disruption: evidence from zebrafish

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Retinoic acid (RA) signalling plays a conserved and fundamental role in vertebrate embryogenesis, and its disruption can lead to developmental toxicity with ecological and population-level implications. Although several AOPs addressing retinoid signalling have recently emerged, mechanistic links between receptor overactivation and defects in sensitive progenitor populations, i.e. neural crest cells (NCCs), remain underrepresented. NCCs are multipotent, migratory population essential for craniofacial, ocular, connective-tissue, cardiovascular and peripheral nervous system formation, placing them at the centre of developmental processes vulnerable to RA signalling disruption. Understanding NCC-specific impacts is critical for improving predictive assessment of chemicals interfering with RA signalling pathway. This study investigated (1) how NCC impairment contributes to RA-induced developmental defects and (2) identified early transcriptional biomarkers. Sox10:eGFP (NCC-marker) zebrafish were exposed from 3 hpf to ATRA (RAR agonist), BMS493 (RAR inverse agonist), UVI3003 (RXR antagonist), or diclazuril (RAR/TR antagonist), alone or in co-exposure. NCC migration pattern was assessed by time-lapse fluorescence microscopy (11-13 hpf), and NCC abundance by FACS isolation of sox10:eGFP⁺ cells at 19 hpf. Transcriptional responses were characterised via RNA-sequencing and Gene Set Enrichment Analysis. Craniofacial and ocular morphology was evaluated in 5 dpf wild-type larvae. RAR overactivation altered early NCC migration patterns and increased cell abundance. Affected pathways included cranial skeletal development, heart valve morphogenesis, connective-tissue organisation, epithelial tube formation, and forebrain regionalization. Co-exposure with BMS493 or diclazuril fully rescued transcriptional and cellular effects, enabling the identification of key NCC-related genes. Corresponding ATRA-induced morphological defect (craniofacial malformation, microphthalmia, retinal pigment epithelium thinning) were also rescued, directly linking RAR overactivation to NCC impairment and its downstream outcomes. Together, these findings establish NCCs as a central and conserved developmental target of retinoid signalling disruption. The resulting NCC-linked transcriptional signatures represent promising biomarker candidates and support the development of mechanistically anchored predictive assays for assessing chemicals that interfere with retinoid signalling.

1.04.T-02 From Theory to Practice: Employing EcR Agonism AOP for In Vitro Screening Assay

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Advances in molecular biology, in vitro assays, and computational modelling are transforming Environmental Risk Assessment (ERA) by providing mechanistic data that enhances our understanding of chemical effects across biological levels. Traditional ERA approaches have largely relied on in vivo studies assessing apical endpoints in a few representative species. A drawback is that such studies provide limited insight into underlying molecular mechanisms and the extrapolation of results to ecosystems is challenging. To address these limitations and align with evolving regulatory and ethical goals to reduce animal testing, New Approach Methodologies (NAMs) enable a more mechanistic understanding for next generation ERA. Integrating in vitro and in silico methods within Adverse Outcome Pathway (AOP) frameworks allows mechanistic data to be connected to higher level biological outcomes, supporting predictive and quantitative hazard assessments. To contribute to this shift, we have developed an in vitro assay based on the Ecdysone Receptor (EcR) Agonism AOP (AOP4) using *Drosophila melanogaster* embryonic (S2) cell line. Ecdysone is an insect hormone regulating development and metamorphosis. However, since EcRs are conserved and thus found in non target species such as pollinators, understanding receptor mediated responses is crucial for a robust hazard assessment. We characterise gene expression responses to the natural ligand for several early key events in AOP4 and to several synthetic EcR agonists for short term and continuous exposure scenarios. This confirmed the initial key events of AOP4 in a concentration and time dependent manner. Utilising molecular docking simulations, we explore how chemical structure influences EcR binding affinity and

activation dynamics, linking receptor level perturbations to transcriptional and functional responses. These mechanistic insights strengthen AOP development and inform model parameterisation for in vitro–in vivo and cross-species extrapolation. Together, we show how combining experimental and computational approaches advances the integration of molecular evidence into predictive ERA frameworks and the early screening of chemicals. Using this approach, it is possible to improve biological realism in predictive ecotoxicology in a mechanistic and ethically sustainable manner, ultimately improving precision and robustness of ERA.

1.04.T-03 Integrating point of departure and structural equation modelling to AOP development: A case study of diuron toxicity in microalgae

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Adverse Outcome Pathways (AOPs) and their quantitative extensions (qAOPs) provide structured frameworks to understand toxicological mechanisms, thereby supporting risk assessments. However, quantitative data for key event relationships (KERs) are often limited. This study used multiple points of departure (POD), including No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), Benchmark Dose (BMD) and half-maximal effective concentration (EC50), combined with structural equation modelling (SEM) to verify and quantify the causal relationships within AOP frameworks. The case study involved *Chlamydomonas reinhardtii* exposed to Diuron, analysis of dose-response data for PSII inhibition, photosynthesis, mitochondrial membrane potential (MMP), ATP content, and growth at different exposure durations. Most endpoints exhibited clear dose-response patterns, with sensitivity increasing over time. A conceptual AOP was developed based on the new experimental evidence and supporting literature. As expected, BMD-based PODs of molecular events (e.g., PSII inhibition) occurred at lower concentrations and earlier than downstream events such as MMP, ATP and growth. The SEM verified the proposed AOP and quantified the KER to support qAOP construction. Additionally, the SEM identified supplementary mechanisms, such as MMP disruption, contributing to growth inhibition. This work proposes a hybrid framework combining POD-based analysis and SEM to strengthen qAOP development.

1.04.T-04 Multi-level Analysis of effects in *D. leopardus* male tree frogs of Fukushima prefecture - Towards fueling the ‘repro-toxicity cAOP’ with wildlife responses.

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The growing release of persistent pollutants in the environment has increased the need to better assess effects on wildlife. To do so, Adverse Outcome Pathways (AOPs) aim to describe a sequence of causally linked events, called Key Events (KE), from the Molecular Initiating Event (MIE) to an Adverse Outcome (AO) at a relevant biological scale, usually population for the Ecological Risk Assessment (ERA). While most of AOP included in AOP-Wiki are based on model organisms, with much of the knowledge acquired at the individual or sub-individual level (a few extending to a population effect), it is nevertheless important to consolidate these patterns of adverse effects on wildlife remaining in a contaminated environment over several generations, and undergoing long-term chronic exposure.

While in 2022, a consensus AOP through six species attempted to highlight the main steps of radio-induced reprotoxicity from laboratory experiments, our study proposes to analyze the biological reproductive responses of male tree frogs during the breeding season, across a dose gradient in Fukushima. Three biological levels, i.e. molecular (RNAseq analysis), cellular (Transmission Electron Microscopy analysis) and individual (sperm parameters such as motility and sperm density) were analyzed on adult testis samples, in order to describe the current observable effects attributable to long-term exposure to radionuclides following the Fukushima accident, and draft a putative ‘radio-induced reprotoxicity’ AOP from in natura derived data.

The results observed in frogs from radio-contaminated areas underline key events already highlighted by the consensus AOP drafted in 2022. More specifically, DNA damage response, oxidative stress and sperm count decrease for males were correlated to the radiation dose. Importantly, these effects, were robust even by controlling for genetic lineage, considered as a confounding factor.

This study highlights that the application of multi-scale experiment on a wild species was able to detect ongoing functional changes on reproductive parameters of a wild species. This comprehensive study on tree-frogs from the Fukushima prefecture is particularly interesting as it enables to fuel a consensus AOP already established with different other taxa. If the results of our work remain correlative, they demonstrate that integration of

molecular analyses with functional assessments can be developed on wild species, increasing the ecological relevance of AOP.

1.04.T-05 Adverse Outcome Pathway Network-Based Approach for the Evaluation of Thyroid Hormone System Disruption for Human Health and Environmental Regulatory Hazard Assessment

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Thyroid hormones (THs) regulate growth, development, and metabolism across vertebrates, making thyroid hormone system disruption (THSD) a critical concern for human and environmental health. Regulatory identification of endocrine disruptors (EDs), such as chemicals inducing THSD, requires evidence of adversity, endocrine activity, and a biologically plausible link, increasingly supported by adverse outcome pathways (AOPs). Over 50 THSD-related AOPs are available/under development (with 7 AOPs endorsed and published by the OECD), forming a cross-species AOP network that underpins mechanistic understanding and informs Integrated Approaches to Testing and Assessment (IATAs). The OECD Conceptual Framework (CF) guides ED assessment by structuring relevant methods into five Levels from *in silico* to *in vivo*. Important challenges remain in the availability and regulatory application of methods for THSD assessment. Notably, no validated *in vitro* assays are currently available at CF Level 2 for THSD mechanisms. In parallel, environmental ED assessment currently relies on amphibian testing, as fish assays capable of adequately addressing the thyroid (T) modality are still lacking at CF Levels 3-5.

In the framework of the European Partnership on the Assessment of Risks from Chemicals (PARC), we collected recent advances in an inventory of 108 methods relevant to THSD, including *in vivo* Test Guidelines, statistically validated *in silico* models, validation efforts for *in vitro* assays, and the validation of thyroid-sensitive endpoints as additions to existing OECD Test Guidelines using fish early-life stages. These efforts align with EU initiatives to phase out animal testing for chemical hazard assessment and implement Next Generation Risk Assessment (NGRA). We propose a modular AOP network-based IATA model integrating mechanistic and effect-level information across CF Levels to support regulatory hazard assessment for both human and environmental health under a One Health framework. In a case study on resorcinol, we demonstrate how emerging methods complement traditional data to strengthen weight-of-evidence for ED classification. By mapping gaps and priorities, this work advances the integration of New Approach Methodologies (NAMs) into chemical safety assessment, supporting a future update of OECD Guidance Document 150 and EU legislative goals for sustainable, NAM-based testing strategies.

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1.04.P Bridging Science and Policy: The Role of Adverse Outcome Pathways in Research and Regulatory Practices to Support Next Generation Risk Assessment

1.04.P-Tu039 Mapping Disease-Linked AOPs to Inform Bioassay Selection for Chemical Hazard Assessment

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Modern societies produce and use thousands of chemicals but most lack appropriate hazard assessment, and likely contribute to the global burden of noncommunicable diseases. Current regulatory toxicity testing is slow, resource-intensive and relies on animal studies focused on apical endpoints. New Approach Methods (NAMs)

offer faster, mechanistically informative, and animal-free testing strategies. However, a systematic selection of biologically relevant endpoints that comprehensively reflects human disease pathways affected by chemical exposures remains a key gap to be filled. Here, we leveraged Adverse Outcome Pathways (AOPs) to identify critical molecular and cellular key events across the six clusters of disease framework – encompassing major noncommunicable diseases linked to chemical exposure. We extracted information from 487 AOPs (AOPwiki, v2024-10-01) and applied filtering criteria, resulting in 284 AOPs manually mapped to the disease clusters. AOP networks for each cluster were constructed, and degree centrality was calculated to assess key events importance within disease networks. The 284 AOPs mapped to one (76%), two (20%), or more (4%) disease clusters, with networks containing 24–90 AOPs. The three most frequently occurring molecular and cellular key events across all clusters were: cytotoxicity (6/6 clusters), oxidative stress (6/6 clusters), and mitochondrial dysfunction (4/6 clusters). These effects are involved in multiple biological pathways and align with oxidative stress being identified as an "umbrella" key characteristic of toxicants. Receptor-mediated key events also showed high connectivity, with aryl hydrocarbon and estrogen receptor appearing in multiple cluster, confirming the importance of receptor-mediated effects and endocrine signalling as toxicants' key characteristics. Other highly connected key events were cluster-specific, such as triglyceride accumulation in metabolic diseases, hence can be informative for detection of specific effects. Building on this analysis, high-throughput cell-based assays covering critical endpoints can be selected, integrating the key characteristics of toxicants framework to ensure coverage beyond current AOP limitations. A panel of 10–15 multiplexed assays meeting criteria for simplicity, robustness, and high throughput could enable efficient screening of single chemicals or their mixtures. Future work will validate this approach by testing diverse chemicals in a selected bioassay panel.

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1.04.P-Tu040 Revealing sublethal effects of *Protoceratium reticulatum* on mussels (*Mytilus edulis*) by adverse outcome pathway (AOP) network approaches

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Harmful algal blooms (HABs) are being reported with increasing frequency worldwide. The bloom-forming dinoflagellate *Protoceratium reticulatum* is a major producer of yessotoxins (YTXs), which have been detected in shellfish globally, yet their toxicity to both shellfish and humans remains insufficiently understood. *Mytilus edulis* plays a key role in marine ecosystems which contributes to marine biodiversity, water quality, and energy flow. Its ecological importance makes it a valuable bioindicator for assessing environmental changes and coastal ecosystem health. Moreover, as a widely consumed seafood species, *M. edulis* represents a direct exposure pathway for marine toxins to enter the human food chain. In this study, we investigated the effects of *P. reticulatum* on *M. edulis* under environmentally relevant bloom conditions. Adult mussels were exposed for six days to diets containing 0% (control), 10%, 50% and 100% *P. reticulatum* supplemented with the non-toxic algae *Tisochrysis lutea* based on carbon-equivalent contents. Transcriptomic analysis revealed that *M. edulis* were significantly affected on gene level. At the individual level, the ingestion rate, O₂ consumption and NH₄⁺ production rate were significantly reduced both for gene-level and pathway level. Benchmark doses (BMDs) were derived at both the gene and pathway levels and integrated with adverse outcome pathway (AOP) networks. Overall, our findings demonstrate that short-term exposure to bloom-level *P. reticulatum* can affect mussel physiological and molecular responses, providing new insights for ecological risk assessment of harmful algal blooms.

1.04.P-Tu041 Early Warning of Aquatic Ecological Risks for Trifluoromethanesulfonimide: Oxidative Stress - Driven Adverse Outcome Pathways and Toxicity Thresholds

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The latent ecotoxicological effects of emerging contaminants (ECs) necessitate the development of early warning systems. Trifluoromethanesulfonimide (TFSI), a lithium battery electrolyte additive, has been recognized as a contaminant of emerging concern. This study established a “mechanism prediction - biological validation - threshold derivation - risk assessment” framework to enable ecological risks early warning for TFSI,

with extensibility to ECs. Network toxicology predicted NFKB1 and PTGS2 as key targets of TFSI, with psoriatic arthritis (PsA) identified as a TFSI-associated disease. By reverse translation of PsA pathogenesis, we predicted adverse outcome pathways (AOPs) of TFSI based on the AOP-Wiki network and screened biomarkers for validation. Cross-species validation in zebrafish models demonstrated dysregulated expression of GCH1/NFKB1/IL34 concomitantly driving dose-dependent oxidative damage and biphasic behavioral alterations, biochemical analysis identified the brain as the target organ. A novel Hormesis model was constructed, which revealed that after correction, EC10 and EC50 resulted in reductions of 90.59 % and 91.37 %, respectively, compared to the traditional model. The combined threshold EC10 overall was derived as 1154.48 ng/L. Risk assessments revealed potential aquatic ecological risks in multiple regions worldwide, a conclusion further supported by risk probabilities quantified through the quantitative adverse outcome pathway (qAOP) model. This work comprehensively uncovered the latent ecotoxicological effects of TFSI, offering theoretical substantiation for addressing regulatory gaps spanning from its initial environmental release to risk manifestation.

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1.04.P-Tu042 AI-Assisted Development of a Comprehensive Adverse Outcome Pathway Network for Oxidative Stress

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Oxidative stress is a central mechanism in (eco)toxicology, linking reactive oxygen species (ROS) formation to cellular dysfunction and adverse organism-level outcomes. Despite its importance, existing Adverse Outcome Pathways (AOPs) capture only parts of this biology, limiting their use for mechanistic assessment, chemical grouping, and interpreting new approach methodology (NAM) data. This work aimed to develop a comprehensive, mechanistically coherent AOP network (AOPN) for oxidative stress-mediated chronic toxicity using a hybrid AI-human workflow that accelerates evidence gathering, integration, and evaluation. An iterative process combined expert oversight with multiple AI tools, including AOP-helpFinder, ChatGPT, and S2CIE. Work began with assembling a conceptual AOPN linking excessive ROS production to reduced growth. AI tools supported initial assessments of biological plausibility and helped refine or harmonize key events (KEs) with those in the AOP-Wiki. A KE-derived search-term library enabled AI-assisted retrieval of relevant literature and mechanistic data from in vitro high-throughput screening resources such as ToxCast. Because searches still produced large literature sets, a second-stage screening of abstracts used AI ranking complemented by expert review. Evidence was evaluated across taxa, including fish, humans, invertebrates, and primary producers. Criteria for the stressor domain were applied flexibly due to limited overlap in stressors across the network. Collected evidence was assessed for biological plausibility, empirical support, and essentiality through a structured weight-of-evidence evaluation performed by human experts. The hybrid workflow increased the speed, consistency, and transparency of AOPN development. AI tools efficiently retrieved mechanistic studies spanning redox biology, mitochondrial energetics, and genotoxicity, while experts resolved inconsistencies, confirmed causal directionality, and ensured alignment with OECD AOP development principles. The resulting oxidative stress AOPN integrates 15 KEs and multiple converging pathways, capturing major processes such as lipid peroxidation, protein oxidation, oxidative DNA damage, and mitochondrial uncoupling. The network reflects established oxidative stress biology as well as under-represented linkages revealed through AI-assisted exploration, providing an expanded mechanistic framework to support regulatory application.

1.04.P-Tu043 Practical Strategies for Translational Modelling-Enabled qAOP Development Using Existing Scientific Evidence

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Quantitative Adverse Outcome Pathways (qAOPs) offer a mechanistic and structured approach for predicting regulatory-relevant adverse effects from molecular and cellular perturbations measured using new approach methodologies (NAMs). A major barrier to developing qAOPs is the limited availability of complete in vivo datasets needed to parameterise quantitative relationships. When such data are incomplete, translating in vitro responses into in vivo key events or adverse outcomes becomes essential and requires robust translational

modelling. This work presents practical strategies and a stepwise workflow for qAOP development that leverages existing scientific evidence rather than relying solely on new experimental studies. The workflow was developed within the EU MERLON project, which aims to enhance quantitative AOP frameworks, support endocrine disruptor assessment and increase the use of human-relevant NAMs. The workflow integrates three core components: (i) systematic data mining and evidence extraction from peer-reviewed literature and in vitro high-throughput screening databases, covering dose-response and time-course data across human and murine systems; (ii) translational modelling using physiologically based toxicokinetic (PBTK)-enabled quantitative in vitro-to-in vivo extrapolation (qIVIVE) to translate in vitro bioactivity into internal concentrations relevant for in vivo key events; and (iii) probabilistic modelling using Bayesian Networks (BN) to represent quantitative key event relationships (qKERs), support qAOP network construction and propagate uncertainties transparently. Initial application shows that, although extensive evidence exists across biological and chemical domains, the information is fragmented, heterogeneous and requires careful evaluation before use in quantitative modelling. Nevertheless, several qualified datasets were identified and used to construct preliminary qKERs. A critical need for dose translation of high-throughput screening data was also identified and will be addressed through targeted qIVIVE for selected prototype chemicals. Overall, this work illustrates practical strategies for making best use of existing scientific evidence in qAOP development. The workflow provides a transparent path for integrating disparate data sources, enabling translational modelling and supporting future quantitative AOP development to advance mechanistically informed risk assessment of endocrine disruptors.

1.04.P-Tu044 Molting inhibition in *Calanus finmarchicus* after exposure to the chitin synthesis inhibitor teflubenzuron

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Chitin synthesis inhibitor teflubenzuron, are widely used in marine aquaculture to control parasitic salmon lice. However, these compounds may also harm non-target species like *Calanus finmarchicus*, a keystone copepod in Arctic and boreal ecosystems. With growing environmental stress from climate change and pollution, it is critical to understand the mechanisms behind such impacts to improve ecological risk assessments.

This study explored the effects of teflubenzuron on *C. finmarchicus*, using Adverse Outcome Pathway (AOP) 360, “Inhibition of chitin synthase 1 leading to increased mortality,” as a knowledge organizing framework. Over a seven-day exposure, we assessed mortality, development, morphology, and gene expression linked to molting processes in the copepod.

Teflubenzuron exposure at 0.012 µg/L caused significant mortality, disrupted development, and led to visible deformities. It also altered the expression of genes involved in molting and chitin metabolism. However, no significant changes in *chs1* gene expression were detected at concentrations between 0.001 and 0.012 µg/L. Thus, we could not directly link *chs1* inhibition to the observed effects as described in AOP 360. Further studies are needed to clarify the molecular mechanisms related to teflubenzuron toxicity and validate AOP 360's relevance for *C. finmarchicus*.

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1.05 Crawling Through Challenges: Emerging Trends in Ecotoxicology for Amphibians and Reptiles

1.05.T-01 Bufadienolide Toxins in Common Toad Skin and Their Link to Population Status

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The European common toad (*Bufo bufo*) is facing gradual population declines likely driven by multiple stressors. As a chemical defence against predators and pathogens, the common toad produces toxins called bufadienolides. Increased toxin production can be considered as a general stress response, as relative toxin

levels were previously found to be increased in populations exposed to anthropogenic stressors, such as environmental contaminants. However, these levels were only assessed in a semi-quantitative way. Therefore, we aimed to develop an analytical method for the quantitative determination of 5 major bufadienolide toxins in the skin of road-killed toads, in order to link population status to differences in toxin levels between populations. A suitable analysis method was developed and validated to quantify 5 bufadienolide compounds in toad skin samples of both non-declining (n=10) and declining populations (n=10) in Flanders (Belgium). As declining populations are assumed to be exposed to severe stressors, we expected to find a correlation between increased toxin levels and population decline. The sampling of road-killed toads was carried out during spring migration (February-March 2024 and 2025) as an alternative to sacrificing live animals from populations which are already facing a decline in abundance. Dorsal skin samples (1-2 g wet weight) were freeze-dried and homogenized using a bead mill. Subsamples (20 mg dry weight) were analysed using a solid-liquid extraction procedure and Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS). A preliminary data analysis of a subset of samples (n=300) indicates that concentrations are slightly, although not significantly, higher in declining populations. Increasing sample size (n=700) will provide a more accurate and representative estimate of the populations and allow for more robust statistical analysis. Differences in toxins concentrations will be linked to population status using generalized linear mixed effects models. Furthermore, we will explore inherent variability due to age, body size and sex, as well as patterns in toxins composition linked to geographical distribution of the populations. The association of bufadienolide toxin levels and population status indicates that toxin levels could potentially be used to identify declining populations. This will aid in prioritizing urgent conservation actions to conserve an ecologically valuable amphibian species.

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1.05.T-02 Preliminary Overview of the Direct and Indirect Effects of Chemical Contamination on Amphibians in the Interface Between Water and Land in Agricultural Landscapes

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Amphibians are the most threatened vertebrate group, one that is uniquely sensitive to the threat posed by chemical contamination, currently one of the main drivers behind the loss of biodiversity. Contributing to this group's vulnerability to chemical contaminants are not only amphibian's particular characteristics, but also the lack of regulation to adequately protect them. Current regulatory efforts are inefficient mainly due to being supported by limited and fragmented Environmental Risk Assessments (ERAs), which is especially alarming in the context of pesticide use in agricultural landscapes for the amphibian species that depend on this habitat. The current ERA framework severely neglects amphibians, not only by not acknowledging them as an individual vertebrate group, but also by contemplating the aquatic and the terrestrial environments in separate assessments, which overlooks the reality of these animals' life cycle, notorious for its flow between both compartments. Furthermore, different chemicals each have their own separate ERA, which also fails to take into account that, in real-life conditions, amphibians can be exposed to varying chemical mixtures by multiple exposure routes. The present work aims to shed light on the risks posed by the flow of pesticides from agricultural lands in central Spain to nearby ponds, for the amphibian populations developing in these ponds; and clarify the role of amphibians as mediators of the return of contaminant's undesirable effects from the aquatic to the terrestrial compartment. After one season (of two) of surveying and sampling food availability, diet composition, susceptibility to diseases, body condition and reproductive output populations of *Hyla molleri*, *Pelobates cultripes* and *Pleurodeles waltl* in eight field ponds, located in the watershed of neighbouring cultivated fields, this study sheds a much-needed light on the general fitness of these animals in the wild. At the same time, it highlights the potential that the conservation of amphibians has, to serve as a tool to break the regulatory silos currently dominating the elaboration of ERAs. Funded by the Horizon Europe project SYBERAC (ref 101135213).

1.05.T-03 Amphibian Body Burden Dynamics: A Refined Mechanistic Model for Dermal Pesticide Uptake from Soil

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Amphibians may be exposed to pesticides in terrestrial scenarios, but at the same time additional animal testing for use in environmental risk assessment (ERA) is discouraged, especially in the context of next generation risk assessment (NGRA). New Approach Methodologies (NAMs) like data-driven in silico approaches can be used to fill this gap. In their terrestrial life stages, the permeable skin of amphibians and their close contact with treated soils suggest that dermal absorption may be a relevant exposure route. Building on the model by Mingo et al. (2024), we present a refined mechanistic toxicokinetic (TK) model that simulates dermal pesticide uptake from soil into amphibians, aiming to provide robust internal concentration estimates and support ERA strategies that do not require additional in vivo testing.

The presented model advances the original one-compartment framework by introducing a two-compartment system that explicitly couples soil and amphibian compartments in a modular approach. It simultaneously integrates key processes such as diffusive and advective uptake, metabolism, excretion, and soil degradation, thereby enabling a dynamic mass balance and more realistic exposure scenarios. For example, body burdens for animals moving in a landscape can now be predicted.

Model validation against empirical data from 815 anurans exposed to 13 pesticides demonstrated robust predictive performance, with 79.8% of predictions conservatively overestimating measured internal concentrations. Sensitivity analyses identified key parameters influencing model output, and scenario simulations illustrated the impact of ecological behavior (e.g., movement patterns) and soil dissipation processes on internal body burdens.

This work advances the mechanistic understanding of dermal pesticide uptake in amphibians, thereby improving future screening approaches and ERA. The refined model offers a transparent and adaptable tool to evaluate amphibian exposure under realistic field conditions, enabling ERA efforts while minimizing the reliance on new animal testing.

1.05.T-04 Impact of multiple stressors on amphibian population dynamics – insights from spatially explicit population modelling

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Amphibians are widely recognized as a vulnerable vertebrate group, yet the drivers of their population declines are complex and multifactorial. While plant protection products (PPPs) often receive considerable attention in ecological risk assessments, other stressors—such as habitat fragmentation, disease, climate variability, and roadkill—can exert equal or greater influence on population viability.

To address these interactions, we developed a spatially explicit, individual-based population model for the common toad (*Bufo bufo*). The model can be applied to address multiple stressors because it integrates ecological and toxicological data, simulates both aquatic and terrestrial life stages, and explicitly represents movement across heterogeneous landscapes. Individuals progress through realistic life phases—from eggs to tadpoles, metamorphosis, juvenile stages, and adulthood—while moving between breeding ponds, summer habitats, and overwintering sites. This structure enables scenario-based evaluations of how landscape configuration and multiple stressors (e.g. traffic) jointly shape population dynamics. Initial simulations demonstrate that landscape structure strongly influences habitat use and spatial distribution. When breeding ponds are surrounded by agricultural areas, individuals disperse widely, whereas proximity to natural habitats promotes concentrated occurrence near ponds. These patterns are likely to affect exposure potential, roadkill and ultimately population dynamics. Simulations showed that a disconnection of the summer habitat and the breeding pond resulted in considerably higher roadkill probability.

Our modeling approach provides a robust framework for assessing cumulative effects of stressors such as PPP exposure, habitat change, and traffic. By capturing spatial heterogeneity and life-history processes, it supports more ecologically realistic risk assessments and informs targeted conservation and management strategies.

1.05.T-05 From Fish to Frogs: Leveraging Fish Toxicity Data to Predict Chronic Pesticide Effects on Aquatic Amphibian Life Stages

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Numerous studies have shown that acute fish toxicity data can predict acute toxicity of pesticides to larval aquatic life stages of amphibians. However, chronic toxicity comparisons have been limited due to insufficient amphibian data. With an expanded database, we compared the chronic sensitivity of aquatic amphibian life stages and fish using apical endpoints from amphibian metamorphosis assays (AMAs) and fish Early Life Stage (ELS) tests for 90 pesticides. Through this comparative analysis, we aimed to derive an interspecies correlation model to predict chronic sensitivity in larval aquatic amphibian life stages based on established fish ELS endpoints.

Apical endpoint data (No Observed Effect Concentrations; NOECs for survival, body weight, and length) were extracted from publicly available registration documents or, in a few cases, from unpublished study reports. Statistical correlations between NOECs (and in addition, between Maximum Acceptable Toxicant Concentrations, MATC) were assessed using Pearson's correlation coefficient. Additionally, AMA responses were compared to their respective counterparts in the ELS test using paired t-tests. For substances where the AMA-NOEC was not directly covered by the respective ELS-NOEC, a stepwise approach was employed to contextualize the results within the EU aquatic risk assessment framework. Using multiple linear regression, we derived an interspecies extrapolation model to predict a chronic surrogate endpoint for larval aquatic amphibian life stages.

Notably, 741% of ELS endpoints were unbound, while 775% of AMA-NOECs were unbound, highlighting the variability in chronic toxicity data. Although the AMA's design limits its use for deriving quantitative NOECs, results indicate that the AMA was equally or more sensitive than the ELS test for most pesticides, with significant correlations observed between the endpoints of both systems. After contextualizing the results within the EU risk assessment framework, the chronic sensitivity of amphibians could be covered for 99% (89/90) of the investigated substances.

The derived interspecies correlation model demonstrated that for 72% of the pesticides, the predicted endpoints were either over-protective or within a factor of three of the observed values, suggesting a reasonable alignment with actual toxicity data. Further validation, considering other pesticides and amphibian species (e.g. from literature data) is planned.

1.05.P Crawling Through Challenges: Emerging Trends in Ecotoxicology for Amphibians and Reptiles

1.05.P-We005 Development of minimally invasive protocols for biomarker detection in amphibians using saliva samples – from laboratory to field application

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Global biodiversity is declining, with amphibians being the most threatened group of vertebrates. Among the key drivers of this decline is exposure to anthropogenic contaminants such as pesticides, heavy metals, fertilizers and pharmaceuticals. Biochemical biomarkers are valuable tools for detecting such exposure and assessing sublethal stresses in both lab and field settings. To measure biomarkers, traditional methods require exposing living organisms to pollutants and rely on invasive sampling—typically resulting in the death of the test subjects. However, in recent years, particular attention has been paid to the 3Rs principle, which aims to replace, reduce and refine animal use in science. In this context, the development of alternative methods to measure biomarkers is essential. As an example, recent studies have successfully explored saliva sampling as a minimally invasive approach for biomarker detection in reptiles. Building on this approach, the present study explores the potential of using saliva as a matrix for biomarker analysis in amphibians under both laboratory and field conditions.

Laboratory work was conducted on African clawed frog (*Xenopus laevis*) and field work was conducted on Swiss populations of alpine newt (*Ichthyosaura alpestris*). Protocols were developed to test three enzymatic biomarkers: EROD, GST and AChE. Liver samples from *Xenopus laevis* were also analysed to compare detection rate with saliva, as this organ is a standard tissue in ecotoxicological assessments. All three biomarkers were detected in liver samples, whereas only EROD and GST could be detected in saliva samples for both

species. The detection rate of EROD and GST was above 90% in *Xenopus laevis* for both saliva and liver samples, whereas it reached considerably lower levels in saliva samples from *Ichthyosaura alpestris*. This pilot study proposes two new minimally invasive protocols to measure EROD and GST activity in amphibians using buccal swabs, contributing to more ethical and sustainable approaches in ecotoxicology. It also highlights the challenge of applying laboratory-developed protocols to field samples.

1.05.P-We006 High-Content Imaging in Amphibian Cell Lines: A Novel In Vitro Platform for Chemical Hazard Characterization

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Amphibians represent the most threatened vertebrate group globally, with chemical pollution recognized as a major contributor to their decline. Yet, despite their high sensitivity, amphibian-specific New Approach Methods for chemical screening are largely lacking. Image-based in vitro assays, such as Cell Painting (CP), are increasingly applied in chemical safety assessment because they detect morphological perturbations across multiple subcellular compartments, enabling the characterization of subtle cellular responses beyond overt cytotoxicity. However, the ecological relevance of such assays is currently limited by the absence of test systems for taxa that are disproportionately affected by environmental contaminants.

Here, we present what is, to our knowledge, the first implementation of the CP assay in two amphibian cell lines, establishing a foundation for expanding high-content imaging to more ecologically relevant taxa. We optimized the assay in two *Xenopus laevis* - derived cell models: carcass fibroblasts (XTC-2) and epithelial kidney cells (A6 CCL) using a 384-well format to enable high-throughput screening. Both lines were exposed for 24 h to a 12-point concentration range of 24 well-characterized CP reference compounds, as well as a set of chemicals with documented in vivo toxicity across diverse amphibian species. Multiplexed staining captured nuclear, RNA, endoplasmic reticulum, Golgi, and cytoskeletal compartments, generating high-dimensional phenotypic profiles. The reference compounds enabled the identification of distinct compound-specific phenotypic signatures.

To evaluate interspecies differences, the same chemical set was profiled in the human A549 lung carcinoma cell line. Potency estimates were comparable between amphibian and human cells for a substantial fraction of active compounds; however, several chemicals - including cytarabine, rapamycin, actinomycin D, and docetaxel - exhibited more than an order-of-magnitude difference in activity across species.

Collectively, these results demonstrate the feasibility of CP in amphibian cell systems and introduce the first amphibian-origin in vitro assay capable of high-throughput screening of chemicals. This work advances the development of ecologically relevant mechanistic test systems and increases the translational value of high-content imaging for environmental risk assessment.

1.05.P-We008 Developmental, Physiological and Metabolic Impacts of Sublethal Delorazepam Exposure in *Xenopus laevis* Tadpoles

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Benzodiazepines, psychotropic drugs, have become a significant group of pharmaceutical pollutants. Their widespread use for anxiety and depression, increasing during the COVID-19 pandemic, has resulted in greater environmental discharge. Wastewater treatment plants cannot entirely remove these compounds, leading to their frequent detection in surface waters. Although developed for human therapeutic purposes, their interaction with conserved GABAergic pathways raises concerns for non-target aquatic vertebrates. Delorazepam (DLZ), a long-acting benzodiazepine, remains ecotoxicologically poorly understood. This study examined the sublethal effects of environmentally relevant DLZ concentrations on early developmental stages of the non-target amphibian *Xenopus laevis*. Tadpoles at Nieuwkoop-Faber stage 46 were exposed for 72 hours to 0.01, 0.1, 1, and 10 mg/L DLZ. Endpoints included mortality, feeding rate, swimming behaviour, growth (body length and weight), heart rate, energy reserves (proteins, lipids, glycogen), and biochemical markers linked to neurotoxicity (acetylcholinesterase, ChE), detoxification (glutathione peroxidase, GST), oxidative stress (catalase, CAT; lipid peroxidation, LPO), and energy production (lactate dehydrogenase, LDH; electron transport system activity, ETS). Exposure to 10 mg/L DLZ caused 85% mortality. At lower concentrations, physiological and biochemical parameters showed dose-dependent changes, but overall survival was not significantly affected. Heart rate and weight remained stable despite reduced feeding rate. Energetic reserves were depleted at 1 mg/L, indicating increased metabolic costs; this is supported by elevated ETS and LDH activities, reflecting enhanced aerobic and anaerobic energy production. Increased antioxidant and detoxification enzyme activities suggest activation

of cellular defence mechanisms. Regarding behaviour, DLZ altered swimming activity: total distance travelled increased while time spent swimming decreased, resulting in higher speed. This may be related to increased ChE activity, which indicates neurotoxicity, although the changes were not statistically significant. The results indicate that DLZ affects various biological processes in *X. laevis*, including behaviour, energy metabolism, and redox balance, even at low concentrations. These findings emphasise the ecological dangers that benzodiazepines present to amphibians and underscore the necessity for better pharmaceutical wastewater management.

1.05.P-We009 Benchmarking Bio-Based DGEVA Versus DGEBA: Developmental and Behavioral Toxicity in Amphibian Larvae

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Epoxy resins are widely used in coatings, adhesives, and composites worldwide, with bisphenol A diglycidyl ether (DGEBA) being one of the most commonly used precursors. However, concerns regarding bisphenol A (BPA) derivatives, including environmental persistence and endocrine disruption, have led to the search for bio-based alternatives. Diglycidyl ether of vanillyl alcohol (DGEVA) is a promising candidate derived from lignin-based monomers; however, its ecotoxicological effects remain poorly characterized. Diglycidyl ether of vanillyl alcohol (DGEVA), derived from lignin-based monomers, is a promising candidate, but its ecotoxicological effects remain poorly characterized.

In this context, the present work aimed at a comparative assessment of the toxicity of the commercially available DGEBA and the bio-based monomer DGEVA in *Xenopus laevis* tadpoles, to evaluate the potential of bio-based resins as safer alternative. Tadpoles were exposed to a range of nominal concentrations (0.79 to 100 mg/L, with a dilution factor of 2,) of each compound. Assays were carried out for 72 h, at 23 ± 1 °C in the dark with continuous aeration. Mortality, body weight, growth rate, and feeding activity were measured as key developmental and behavioral endpoints.

The results indicated that DGEBA caused higher mortality than DGEVA; the former compound induced 100% mortality, after 24 h of exposure, at concentrations of 100 and 50 mg/L. The LC50 and LC20 values were for 10.4 and 6.7 mg/L DGEBA and for 29.9 and 17.7 mg/L DGEVA, respectively. No significant differences were found in weight, despite the reduced feeding rate at the lowest concentrations (0.78 mg/L in DGEBA and 0.78 and 1.56 mg/L in DGEVA). Relatively to morphometry, tadpoles showed no significant differences relative to the control, except for a significant increase in total length and body width at 0.78 mg/L in DGEBA, and an increase of total and tail lengths at 25 mg/L of DGEVA.

Overall, these findings contribute to understanding the potential trade-offs of substituting conventional petroleum-based epoxies with bio-based analogs.

1.05.P-We010 Effects of dermal exposure to pesticides on skin structure and function of amphibian terrestrial stages

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Amphibians are especially susceptible to dermal exposure to plant protection products (PPP) because they lack tegumentary protective that limit the uptake of these substances. Amphibian skin plays diverse physiological functions, so the direct contact of PPP with it may not only result in a quick absorption, but also come along with direct toxicity through effects on those functions. The aim of this study was to determine how dermal exposures of amphibian post-metamorphic stages to PPP active ingredients and formulations resulted in pesticide accumulation in the skin and affected its structure and physiological, namely immunological, function. We exposed post-metamorphic Iberian waterfrogs via overspray to six active ingredients (a.i.) or their formulations, using two doses: the labelled spray application rates and an increased rate that varied between 3 and 10 times the labelled rate depending on the a.i. solubility. Skin secretions were collected after seven days to quantify concentration and antimicrobial capacity of skin peptides. Exposure to the flupyradifurone formulation Sivanto Prime lowered peptide concentrations and tended to reduce their ability to inhibit the growth of a pathogenic *Aeromonas caviae* bacteria. Also at day 7 post exposure, froglets were euthanised to measure pesticide residues in skin and conduct a histological assessment of the epidermis at the lateral plicae. Flupyradifurone, azoxystrobin and tebuconazole formulations led to higher PPP accumulation in froglet skins than a.i. The epidermal index indicative of skin thickness was increased after exposure to azoxystrobin and α -cypermethrin, with stronger effects caused by formulations, whereas effects of metrafenone and fluzifop-p-butyl on this

index were detected only after the exposure to the a.i.. The results of the study evidence that formulations play a major role in the effects of PPP on amphibian skin following overspray, that lipophilicity drives effects on epidermal histological structure and that some substances may compromise the immune function of the skin by reducing both the total amount and the antibacterial efficacy of skin peptides.

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1.05.P-We011 Why Size Matters: Clarifying the Calculation of Anuran Body Surfaces

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Amphibians have a moist and permeable skin, enabling exchange of gases and water with the environment, while also acting as a potential route of exposure to environmental contaminants. Therefore, the correct estimation of surface area for amphibians is highly relevant for estimating exposure to contaminants. Recently, some proposals have been made to address risk of pesticide exposure to amphibians, typically focusing on anurans (frogs and toads) as most widely distributed and occurring in agricultural landscapes. A publication often used in these proposals is Hutchison et al. (1968), titled “Relation of body size and surface area to gas exchange in anurans”, describing the respiratory function of amphibian skin. In their publication, the authors measured the surface (skin) area of different anuran species and derived an allometric relationship to body weight. The equation has been incorrectly applied in a number of scientific and regulatory documents about pesticide exposure of anurans.

When comparing results of different equations that are proposed to estimate pesticide overspray area of frogs and hence their exposure, we noted up to 10-fold differences in results that were difficult to explain. Also the allometric equation had been applied without considering the log scale for surface area (SA in cm²) and body weight (bw in g) as originally published.

This work highlights and aims at correcting a repeated error in different scientific and regulatory publications assessing amphibian exposure to pesticides, e.g. by overspray during pesticide application. Incorrect application of the Hutchison et al. (1968) equation (i.e. failing to de-logarithm the first term of the equation) leads to a ca. 10-fold underestimation of total surface area and a 3-fold underestimation of dorsal surface area of anurans. To estimate anuran dorsal surface area explicitly in pesticide overspray exposure assessments we recommend using the simpler Weltje et al. (2017) ellipse equation (which could also be used as a starting point for ventral contact with soil) as a practical approximation.

1.06 Crossing Scales in (Eco)toxicology: Using Multi-Omics to Understand and Predict Physiological and Ecological Responses

1.06.T-01 Connecting the Dots: data-driven clustering of mechanisms of action based on molecular signatures of gene expression profiles in zebrafish embryos.

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In order to utilize the potential of recently accessible OMICs data in the context of ecotoxicology, this study employs the connectivity mapping (Cmap) approach on 32 chemical substances, belonging to 10 different modes of action (MoAs) in zebrafish embryos. The 32 chemicals were clustered by their transcriptome profiles. Furthermore, a comprehensive strategy was formulated for the execution of Cmap on zebrafish embryo transcriptome data, encompassing the evaluation of the performance of various Cmap models. The vector-based scoring (Pearson's correlation) revealed overall higher accuracy than the aggregation-based scoring (GSEA). Additionally, higher accuracy was achieved using a smaller gene input size in Cmap. Furthermore, GSEA was performed to map the chemical substances at the functional level and inspect the functional signatures of the given MoA. Finally, a random forest model was applied to the transcriptome profiles of *Danio rerio* exposed to chemicals, to define unique molecular signatures for each MoA. This study represents a significant step towards integrating the existing knowledge of single molecular responses with the objective of deriving data-driven

biological conclusions about the ecotoxic MoA of chemicals. Additionally, the defined molecular signatures provide a potential cost- and time-effective MoA prediction strategy.

1.06.T-02 Spatial Multi-Omics to Advance Toxicity Assessment of Tributyltin in Zebrafish Embryos

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Tributyltin (TBT), a widely used antifouling agent, remains a pervasive marine pollutant with endocrine-disrupting and neurotoxic properties. To elucidate early neurotoxic mechanisms, zebrafish embryos (*Danio rerio*) were exposed to environmentally relevant TBT concentrations (3?nM and 30?nM) for 24?h at 4?dpf. A spatial multi-omics framework integrated MALDI-MSI lipidomics, MERFISH-based transcriptomics, and phenotypic assays. Cryosectioned larvae (7?µm) underwent MALDI-MSI lipid profiling at 15?µm resolution using timsTOF fleX, followed by multivariate analyses. Spatial transcriptomics employed MERSCOPE® with a 1,000-gene panel, clustering via Seurat. Phenotypic endpoints included LC-MS/MS neurotransmitter quantification, behavioral assays (escape and visual motor responses), and chromatic preference tests. TBT induced dose-dependent lipid disruptions in brain, eye, and tail regions, predominantly affecting glycerophospholipids (>60%), alongside sphingolipids and sterols. Approximately 20–25% of lipids showed coordinated dysregulation, more pronounced at 30?nM. Transcriptomic analysis identified 36 cell clusters and 218 DEGs (FDR?<?0.05) in neural tissues, largely downregulated and linked to neuronal communication, neurodevelopment, lipid metabolism, and stress pathways. Notochord DEGs implicated morphogen signaling, reinforcing CNS patterning disruption. Multi-omics integration revealed impaired neuronal differentiation, myelination, and activity-dependent signaling, with lipid remodeling (PC, PE, DG), sterol/HexCer accumulation, and Ca²⁺-linked stress converging with apoptosis activation. Behavioral deficits, altered color preference, and monoaminergic neurotransmitter imbalance confirmed functional consequences. TBT disrupts zebrafish neurodevelopment through coordinated molecular and phenotypic alterations. Spatial multi-omics uncovered lipid remodeling and neurodevelopmental gene downregulation validated by behavioral impairments. These findings underscore the value of advanced spatial omics in environmental hazard assessment and regulatory frameworks.

Disclaimer/Disclosure:

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1.06.T-03 Meta-Metabolomics to Unravel Biopesticide-Induced Structural, Functional and Adaptive Responses in Benthic Microbial Communities

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Faced with growing concern about the environmental and health impacts of pesticides, biopesticides emerge as a less hazardous alternative. Nevertheless, there is paucity of knowledge about their actual effect on non-target species. In particular, the response and further tolerance of microbial communities—key actors in aquatic ecosystem structure and functioning—to these « natural » chemicals remains poorly understood. To address this gap, meta-metabolomics is a cutting edge approach by providing a snapshot of the molecular/biochemical responses of microbial communities exposed to biopesticides.

This study aims to elucidate the responses and tolerance mechanisms of leaf-associated microbial communities exposed to kasugamycin, a biosubstance with antifungal and antibacterial properties. To this end, an untargeted metabolomic approach was combined with functional (enzymatic activity, respiration), structural (biomass) and adaptive (Pollution-Induced Community Tolerance) analyses to map and understand the different responses and

metabolic pathways affected during a 28-day chronic exposure .

Results showed a decline in bacterial density and community respiration, accompanied by an increase in fungal biomass and a stimulation of β -glucosidase activity under kasugamycin exposure. Tolerance to kasugamycin emerged in pre-exposed communities only at day 7. Metabolomic profile analysis revealed significant effects of both time and kasugamycin concentration. The metabolite classes most affected by kasugamycin were peptides and amino acids. These shifts likely confirmed that kasugamycin disrupts protein synthesis, in accordance with its known mode of action on prokaryotes.

This work represents a major step forward in microbial ecotoxicology by applying untargeted metabolomics to benthic microorganisms, providing an integrated picture of exposure and molecular responses at the community level. Metabarcoding will complement these analyses by revealing taxonomic composition and diversity.

Ongoing annotation of metabolites and pathways, along with further investigation of the lipophilic fraction, will help to elucidate the cascade of reactions leading to structural and functional responses and tolerance (i.e. eco adverse/tolerance outcome pathways) acquisition in leaf litter exposed to a biopesticide. Ultimately, this approach may allow the identification of molecular descriptors that could serve as biomarkers to support the monitoring and protection of aquatic ecosystems.

1.06.T-04 Ecotoxicity and mechanism of antifouling paint particles on Pacific white shrimp

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The antifouling paint particles (APPs) have emerged as one of the microplastics. But there are limited studies of APPs on living organisms, so this study aimed to evaluate the ecotoxicity and toxicological mechanism of APPs on aquatic ecological receptor. Pacific white shrimp (*Penaeus vannamei*) were exposed to APPs collected from the hydro-blasting effluent of dry-dock and toxicological symptoms and mechanism were analysed. *P. vannamei* got growth inhibition and changes in molting patterns. The organ damages (exoskeleton, hepatopancreas, and intestine) were observed. By using multi-omics analysis, we found that APPs induced disturbances in membrane transport and amino acid metabolisms, which eventually caused irreversible disability of tissue-organism level on shrimp. As *P. vannamei* are valuable ecological receptors and food source for human, this result suggests the potential risks of APPs on marine ecosystems and needs for food source management from APPs contaminations.

Disclaimer/Disclosure:

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1.06.P Crossing Scales in (Eco)toxicology: Using Multi-Omics to Understand and Predict Physiological and Ecological Responses

1.06.P-Tu046 Developing Non-lethal Bio-dosimetry Techniques Using Fathead Minnow (*Pimephales Promelas*) Proteomics and Metabolomics

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With the looming threat of climate change, governments around the world have begun investing in alternative energy sources to replace fossil fuels, with a significant emphasis on nuclear energy. Small modular reactors (SMRs) can replace fossil fuel usage. In addition, they can be used in small, remote communities for electricity generation, desalination, and heating. However, while still novel, there is limited knowledge of the effects that SMRs might have on their surrounding environment. While there is literature on the effects of ionizing radiation to fish, most studies have focused on acute, single-exposure developmental toxicity above 1 Gy, with limited results on the effects of lower levels over long-term, chronic exposures. Our study aims to fill that literature gap by investigating the effects of ionizing radiation on the proteome and metabolome of the fathead minnow (*Pimephales promelas*). We will sample across multiple tissue types, including mucus, plasma, gill, skin, and muscle. We expect to see changes in protein concentrations related to reactive oxygen species, pigmentation and sex hormones. These results can be used to develop non-lethal bio-dosimetry programs for the surrounding environment of SMRs, enabling consistent monitoring while reducing stress on sample organisms and providing additional data for risk assessors.

1.06.P-Tu047 Non-Invasive Biofluid Profiling for Detecting Low-Dose Radiation Exposure Using Omics Analysis

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To mitigate carbon emissions and meet global energy needs, nuclear power has increasingly been suggested as a complementary solution in the pursuit of low-carbon energy sources. Among the new facilities and technologies to be soon operational are small modular reactors (SMRs). They are designed to be pre-made and transported for installation close to industrial facilities, as well as in remote areas, such as lakes and the ocean. Therefore, exposure to low-dose radiation is likely to occur in a range of species, including fish and humans. In this study, we aimed to establish alternative non-invasive biodosimetry approaches using omics analysis. For the fish component of the study, we exposed fathead minnows to low and high ionizing (gamma) radiation for periods that would result in doses of 0.1 and 1 Gy, respectively. For the long-term 0.1 Gy exposure, we used coin-sized Cesium-137 sources with fish exposed for 7 days. Meanwhile, a gamma ray irradiator system was utilized for the higher dose of 1 Gy over a period of 2 hours (shorter term). Fish mucus samples were collected with paper tissues and analyzed for protein and metabolite biomarkers using untargeted mass spectrometry (MS)-based analysis. We also collected human saliva from radiation workers (potential exposure group), administrative workers (negative control) and patients undergoing radiation-based diagnostic procedures (positive control). The saliva samples were processed, and quantification of proteins and metabolites was performed using the same MS-based approaches as for fish mucus. Bioinformatics tools will be used for the identification of biomarkers of exposure as well as for understanding impacts on specific pathways. We predict that responsive biomarkers will be associated with DNA damage as well as oxidative stress responses. Given the potential of an increase in low-dose radiation exposure for both wildlife and humans, the identification of biomarkers of exposure could strengthen routine health monitoring and screening programs for nuclear sector workers, as well as improve environmental monitoring efforts.

1.06.P-Tu048 Slippery Science – Fish Mucus for Non-invasive Biomonitoring

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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 barrier, fish epidermal mucus plays a critical role in its 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 constantly, allowing for its non-invasive collection with relative ease compared to other methods (i.e., blood and tissue sampling).

We aimed to develop methods to identify and measure proteins in fish epidermal mucus, for low-impact monitoring of threatened and/or protected wild fish populations. We optimized two sampling methods that can be used in lab and remote field environments. We also characterized the types of proteins we could find in mucus to better understand its practical application for fish health studies. We refined our collection methods to work on different species and sizes of fish that occupy different environments.

All mucus, plasma, and tissue samples were analyzed using liquid chromatography tandem high resolution full scan mass spectrometry (Agilent 1260 Infinity LC with 6545 QTOF), and data were processed using Agilent Spectrum Mill (Version B.04.01.141), Skyline 20.2 (MacCoss Lab Software), and Metaboanalyst 5.0 software. We will present the results from our method development and optimization, and then compare mucus proteins from four species of fish (Rainbow trout, Lake sturgeon, Fathead minnow, and Bluntnose dace). Finally, we will demonstrate how the mucus proteome adequately informed our understanding of the toxicological effects from three different laboratory exposure studies (Ni²⁺, Benzalkonium chloride, and PFAS).

1.06.P-Tu049 Investing Gut-Brain Axis disruption and Neurotoxicity Following Nanoplastic Exposure in Adult Zebrafish (*Danio rerio*)

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Nanoplastics (NPs) are increasingly recognized as common contaminants in aquatic ecosystems, where they can accumulate in organisms and affect multiple physiological systems. The gut-brain axis (GBA), which links gut microbial composition and intestinal barrier function to central nervous system activity, is thought to be highly sensitive to environmental stressors. However, the mechanisms by which chronic NP exposure disrupts gut homeostasis and contributes to neurotoxicity through the GBA remain insufficiently understood.

This study aims to explore whether NP-induced gut dysbiosis contributes to neurotoxicity via the GBA in adult

zebrafish (*Danio rerio*). Adult male zebrafish are being exposed to 1 mg/L NPs under controlled laboratory conditions. Following exposure, behavioral assessments are conducted, after which intestinal tissues, gut contents, and brain samples are collected for molecular and microbiological analyses. Gut microbiota composition is assessed using 16S rRNA sequencing, while RT-qPCR and ELISA will be used to evaluate intestinal barrier gene expression, neuroinflammatory markers, and related protein mediators. We hypothesize that NP exposure will shift gut microbial composition, weaken intestinal barrier function, and trigger neuroinflammatory responses, leading to changes in behavior. This work is expected to provide mechanistic insights into how NP exposure affects systemic health, emphasizing the role of the GBA in linking gut disturbances to neural outcomes. These findings may inform ongoing discussions in ecotoxicology and public health regarding the impacts of micro/nanoplastic pollution.

1.06.P-Tu050 Brain Metabolic Responses of Honey Bees (*Apis Mellifera*) Exposed to Coumaphos and Fipronil Using UPLC-Q-TOF/MS

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Honey bees (*Apis mellifera*) serve as keystone pollinators essential for agricultural productivity, biodiversity maintenance, and overall ecosystem stability. However, recent global declines in honey bee abundance and diversity have raised substantial concern, with the widespread use of pesticides in agricultural and natural ecosystems identified as a major contributing factor. Among these pesticides, coumaphos, a synthetic organophosphate used to manage Varroa mite infestations within colonies, and fipronil, a phenylpyrazole employed to control agricultural pests, have both been shown to adversely affect honey bees. Evidence indicates that coumaphos exposure can detrimentally impact honey bee physiology and behavior, including increased cellular damage, impaired olfactory learning, and reduced foraging activities. Similarly, fipronil disrupts bees' communication and foraging activity, ultimately increasing colony vulnerability.

In the present study, honey bee colonies were provided with feeding solutions containing coumaphos or fipronil for either 1 day or 10 days to examine the effects of each pesticide. The exposure concentrations of coumaphos and fipronil were determined based on literature surveys identifying concentrations detected in pollen and honey within colonies. Both pesticides had negative effects on honey bee nursing performance over 10 days of exposure, including reductions in the number of bees and the duration of nursing behavior. To better understand nursing behavior changes in bees exposed to each pesticide, we conducted comparative metabolic profiling of honey bee brains following exposure to coumaphos or fipronil using UPLC-Q-TOF/MS.

The results revealed that the levels of 73 and 69 metabolites were altered after exposure to coumaphos and fipronil, respectively. Among these altered metabolites, coumaphos significantly affected key metabolites related to NAD⁺-dependent signaling, whereas fipronil exposure caused changes in metabolites involved in arginine and proline metabolism. These findings provide new insights into the potential mechanisms through which individual exposure to coumaphos or fipronil disrupts nursing behavior in honey bees, underscoring their risks to colony health.

1.06.P-Tu051 Comparative Toxicometabolomics and Proteomics of Insecticide Abamectin in Honeybee

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Abamectin, an insecticide, has been used widely to control mites, nematodes, and various insect pests in crop. In order to investigate the toxicological mode of action of abamectin on insects, the honeybee was selected as the representative insect, utilizing an integrated multi-omics approach combining targeted metabolomics (GC-MS/MS) and toxicoproteomics (LC-HRMS). Honeybees were exposed to sub-lethal concentrations of 0.115 ng/uL for the low-exposure group and 1.15 ng/uL for the high-exposure group based on the 48-h LD50 value (0.0023 ug/bee) under controlled conditions.

For metabolomics, samples were extracted with 80% methanol and the extract was prepared with TMS derivatization for GC-MS/MS. The targeted MRM mode detected 239 metabolites, and non-targeted LC-HRMS was also used. Statistical analysis (PLS-DA, VIP, ANOVA) identified biomarkers. Pathway analysis (MetaboAnalyst 5.0) revealed significant disruption in core functions: Citrate Cycle (TCA cycle), Tyrosine metabolism, Phenylalanine/tyrosine/tryptophan biosynthesis, Alanine/aspartate/glutamate metabolism, and Ascorbate/aldarate metabolism. For proteomics, proteins were extracted with 0.1 M phosphate buffer and digested using the FASP method. LC-HRMS identified 670 proteins, and 32 proteins were selected as significant biomarkers through volcano plot. The integrated multi-omics evidence confirms that abamectin

exposure perturbs fundamental metabolic and biochemical networks in honeybee. The omics study proves that it can identify the mode of action and toxicological mechanisms in detail over simple LD50 values.

1.06.P-Tu052 Assessing the Impact of Fluorescent Polyethylene Terephthalate and Phenanthrene on the Blue Mussel by Omics Approaches

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Microplastics (MPs) are a threat to aquatic life in part because they can act as carriers of pollutants by adsorbing them to their surface and contributing to bioaccumulation. Environmental metabolomics could offer meaningful information on the biochemical response to exposure to evaluate the impact of multi-contamination on marine biota without any a priori information. Hence, the goal of this study was to investigate the accumulation and effects of single and combined exposures on metabolomic signatures in the marine bivalve *Mytilus edulis*. Blue mussels were exposed for 7 days to PET mixture with sizes of (300-500 µm, 150-300 µm, 80-150 µm and 40-80 µm), with and without the polycyclic aromatic hydrocarbon (PAH) phenanthrene (Phe) (0.5 mg/L), which is dominant component of crude oil. PET was quantified using Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS); Phe was quantified using GC-MS both were found to accumulate at higher levels in digestive gland (DG) tissue compared to other organs haemolymph (HL) and gill. We investigated the metabolic profile of HL and DG using non-targeted metabolomics through the utilisation of GC-MS and liquid chromatography-mass spectrometry (LC-MS). Between exposed and non-exposed groups, we identified 35 distinct metabolites in mussel HL by GC/MS, and in the DG we used a combination of GC-MS/LC-MS and found 80 and 75 distinct metabolites, respectively. We applied univariate (ANOVA $P < 0.05$), and multivariate (PCA and PLS-DA/sPLS-DA) statistical analyses on the data sets. We identified the metabolites that drive the differences across treatment groups in HL, namely uracil and citric acid. In DG, the most unique biomarkers were glycolic acid and taurine metabolites revealed by GC-MS, and 1-Hydroxy-2-Naphthoate metabolites detected by LC-MS. Our study demonstrates the suitability of metabolomics analysis to reveal metabolic perturbations in the HL and DG tissues of *Mytilus edulis* following exposure to environmental toxicants. Integrated HL-DG metabolomics (GC-MS and LC-MS) revealed a coherent biomarker panel through sPLS-DA, with the combined-exposure group exhibiting the strongest metabolic enrichment and the most distinctive biomarker profile. These findings offer a better understanding of the impacts of combined PAH and MNPs pollution on the health of blue mussel populations from pollutants.

1.06.P-Tu053 Assessing the linkages between Vitellogenin Amino Acid and Protein Sequences to Selenium Developmental Toxicity Thresholds among Fish

Theresa Warriner and Denina B.D. Simmons, Ontario Tech University, Canada

Vitellogenins are a family of lipo-proteins found in females in many oviparous species. Synthesized in the liver, vitellogenins are then transported to unfertilized eggs, where they are cleaved into multiple, smaller egg yolk proteins to prepare for egg fertilization. As a result, the transfer of vitellogenin from female to offspring can be a pathway in which environmental substances can influence egg and progeny quality. Selenium is an essential element that is required in low concentrations for the amino acid selenocysteine (by substituting selenium for sulphur in cysteine). However, when organisms have an excess of selenium, selenomethionine becomes abundant (by substituting selenium for sulphur in methionine), which can negatively impact protein sequence, structure, and function. Studies investigating selenium toxicity have shown that parental exposure in fish can lead to decreased hatching rates and increased offspring mortality and developmental deformities. We propose that vitellogenin may be a vehicle of selenium into unfertilized eggs, and that the selenium toxicity thresholds in a species may be regulated by the abundance and sequence of vitellogenin and other egg proteins. To determine whether vitellogenin amino acid and protein sequences correlate with selenium toxicity thresholds, we used liquid chromatography tandem mass spectrometry (LC MS/MS) to examine the vitellogenin sequences in representative fish species (with varying selenium toxicity thresholds). We will present 1) the abundance of cysteine and methionine in vitellogenin sequences and in other egg proteins, 2) the abundance of vitellogenin isoforms across different fish species and correlate these abundances with established selenium toxicity thresholds. This project may provide evidence for a mechanism for selenium toxicity.

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1.06.P-Tu054 Male-biased gut dysbiosis following environmentally relevant benzalkonium chloride exposure in fathead minnows (*Pimephales promelas*)

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Quaternary ammonium compounds (QACs) are widely used antimicrobial chemicals in household and industrial products. Given their microbial-targeting properties, their release into aquatic environments raises concerns about potential impacts on microbial communities, including the gut microbiomes of aquatic organisms. Yet, the impacts of QACs on these communities remain poorly understood.

To investigate these impacts, we exposed male and female fathead minnows (*Pimephales promelas*) to environmentally relevant concentrations (0.5-15 µg/L) of benzalkonium chloride (BAC), one of the most widely used QACs, for 28 days in flow-through tanks under controlled conditions. Following exposure, fish guts were dissected, DNA extracted, and microbial communities were profiled via amplicon sequencing of the V3-V4 region of the 16S rRNA gene on an Illumina MiSeq platform, generating 300 base pair paired-end reads for a total read length of 600 base pairs per fragment.

Control fish showed no significant sex differences, indicating that baseline gut microbiomes were intrinsically similar. In BAC-exposed males, PERMANOVA analysis indicated a significant shift ($R^2 = 0.22$, $F = 3.01$, $p = 0.007$) in overall gut community composition in response to BAC exposure. In BAC-exposed males, *Enterococcus* (including *E. gilvus*) was significantly reduced, while *Peptostreptococcaceae* showed a significant increase with increasing BAC concentration. *Enterococcus* are facultative anaerobes that produce lactic acid via homofermentative metabolism, supporting gut homeostasis and colonization resistance. *Peptostreptococcaceae* are obligate anaerobes that ferment peptides and amino acids to produce short-chain fatty acids (SCFAs), including acetic, butyric, isobutyric, caproic, and isocaproic acids. The observed shift may therefore alter the balance of fermentation products in the gut, potentially affecting SCFA composition and related microbial functions. In comparison, female gut microbiomes showed no significant changes, reflecting resilience to BAC-induced perturbation, which may be mediated by host-protective mechanisms such as hormonal regulation or immune modulation.

Overall, these findings demonstrate that BAC exposure can induce sex-specific gut microbiome dysbiosis, with males particularly susceptible, which may predispose exposed fish to impaired gut function and negative health outcomes, highlighting the ecological and physiological risks of BAC contamination in aquatic environments.

1.06.P-Tu055 Assessing the Applicability of Sediment Metabolomic Biomarkers for Quantifying Phytoplankton Damage under Chemical Spill Incidents.

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Damage to phytoplankton, the primary producers in aquatic food webs, caused by chemical accidents results in a severe loss to aquatic ecosystems; however, the extent of damage to algae has not been calculated during the regulatory damage assessment process. To address this issue, our previous study simulated chemical spill conditions by exposing algae to river water and sediment contaminated with chemical hexamine. We subsequently applied non-targeted metabolomics using LC-QTOF-MS to analyze the sediments mixed with dead algae. The results demonstrated that biomolecules detected from sediment can reflect population-level damage of algae and even approximate the timing of death. To evaluate the applicability of this methodology, we expanded the experiments to different conditions: two algal species, three chemicals, and three different water stream samples. To find metabolites that indicate algal mortality irrespective of experimental conditions, we performed cluster analysis and pattern hunter based correlation analysis. As a result, we identified approximately 40 tentatively annotated metabolites that consistently exhibited increased or decreased abundance in chemically impacted samples, even when algal species, chemical type, or sampling location differed. Among these metabolites, a lignan glycoside-like compound and an isoquinoline-derived compound exhibited condition-independent responses under all tested scenarios. Therefore, this study, as an extension of our previous work, provides evidence that certain metabolites may serve as broadly applicable, non-algae species-specific, non-chemical-specific, and non-location-specific biomarkers for assessing phytoplankton damage resulting from chemical exposure. The findings presented here demonstrate the feasibility and broad applicability of sediment metabolomics as a framework for evaluating phytoplankton damage under chemical spill scenarios.

1.06.P-Tu056 Multiple stressor effects in freshwater invertebrates – lessons learned from transcriptomics
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Stream ecosystems and their associated biodiversity are threatened by multiple anthropogenic stressors such as pollution, overexploitation and hydromorphological degradation. Traditionally, these stressor effects are quantified at the population level through changes in organismal abundances. While this provides valuable insights in stressor-induced structural alterations of biological communities, stressor effects at the individual level i.e., sublethal effects, are often neglected in multiple stressor studies. However, they may represent early warning signals before biodiversity loss manifests. We used RNA-Sequencing to profile the transcriptomic stress responses in multiple stream macroinvertebrate species exposed to various chemical and physical stressors. Specifically, we aimed to unveil stressor-induced molecular response mechanisms linked to pesticide exposure, fine sediment deposition, reduced discharge and salinization. We found that in several cases, transcriptomic responses to single and combined stressor exposure were in line with previously detected stressor effects at the population level such as the antagonistic interaction between fine sediment deposition and reduced flow velocity in the amphipod *Gammarus fossarum*. At the same time, transcriptomic data revealed stressor effects that would have remained unnoticed when focusing on abundance data alone. Further, we detected stressor-induced mechanisms at the functional genomic level that were unexpected, such as an altered expression of genes involved in development in insect larvae exposed to the insecticide chlorantraniliprole. These findings underscore the complexity of multiple stressor dynamics, highlighting that an improved mechanistic understanding of multiple stressor effects is urgently required. We here demonstrate one possibility to achieve this, namely to combine transcriptomics with ecological experiments, allowing us to synthesize stressor effects that are consistent across different experimental settings, organismic groups and ecological scales.

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1.06.P-Tu057 A Developmental Self-Organising Map of the Zebrafish Embryo Transcriptome
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Chemical risk assessment is constrained by the scale of untested compounds and the limited interpretability of static toxicogenomic designs. Zebrafish embryos offer a tractable vertebrate system, but their rapidly shifting transcriptional states require models that capture developmental context. A developmental reference model was built to characterise transcriptome dynamics across zebrafish embryogenesis. Five RNA-sequencing datasets covering fertilisation to 120 hours post-fertilisation were integrated, yielding more than 250 samples across 49 timepoints. Gene-wise temporal expression was modelled using generalised additive models, capturing non-linear profiles and accounting for inter-study variation. These profiles served as input for a 60×60 self-organising map that arranged genes by similarity in developmental timing. The resulting topology represented coordinated expression waves and produced clusters enriched for discrete biological functions, forming a coherent transcriptional atlas of early zebrafish development. In an initial analysis, we projected a dataset of zebrafish embryos exposed to the angiogenesis inhibitor SU4312 onto the devSOM. Depending on the timepoint of observation, distinct regions of the map are regulated, indicating that chemically induced transcriptional responses are strongly dependent on developmental stage rather than occurring as uniform stress signals across time. The developmental self-organising map therefore provides a framework for interpreting toxicogenomic signatures in the context of embryonic progression, improving mechanistic resolution and supporting comparative analyses across datasets.

1.07 Daphnia- Advances, Problems, & Historic, Novel and Interdisciplinary Approaches

1.07.T-01 Bridging the Gap: Using High-Throughput Ecotoxicological Tests to Facilitate Chemical Extrapolations in the Standard Dynamic Energy Budget Theory for *Daphnia magna*
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Current ecotoxicological endpoints, both in tests and effect modelling, focus primarily on survival and reproduction, forgoing growth due to difficulties in its measurement. However, novel approaches to ecotoxicological testing have demonstrated that the three endpoints can be measured simultaneously in *Daphnia magna*, and potentially other organisms. As these tests differ to standardized approaches due to their experimental design, it is important to understand the observed differences in lethal and sublethal endpoints. To bridge the gap between standardized test results and high throughput techniques, data obtained from high throughput ecotoxicological tests that closely replicate those of the OECD *Daphnia* reproduction test No. 211 were compared to the original data used in the approval process for two neonicotinoid pesticides (Acetamiprid and Thiacloprid). Data for a third neonicotinoid chemical (Imidacloprid) was also available, and an extrapolation of lethal and sublethal endpoints with mathematical reasoning was derived using endpoint data available in literature.

Using the Dynamic Energy Budget (DEB) Standard Toxicokinetic-Toxicodynamic (TK-TD) model, best fits were found for the high throughput data in DEB parameters, modes of action and TK-TD parameters. The original data for the chemicals was then used as inputs for the DEB TK-TD model and the predicted endpoints were compared. Results point to the high throughput model underestimating or overpredicting survival and reproduction in most cases. However, when growth data was available, the predicted endpoint closely matched the original report. For the extrapolation to Imidacloprid, applying a conversion factor to the TK-TD parameters for Acetamiprid and Thiacloprid resulted in the predicted growth and reproduction endpoints closely matching the original ones.

This study highlights the differences in modelling high throughput and regular standardized testing, demonstrating that the observed differences in sublethal endpoints can be explained by DEB modelling. However, for lethal endpoints, more studies are necessary to understand the differences between the two types of testing.

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1.07.T-02 Molecular Docking On *Daphnia Magna* Methoprene Tolerant Receptor As A Predictive Approach For Potential Environmental Endocrine Disruptors

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Molecular docking is a computational method predicting molecular binding modes. This *in silico* approach usable for environmental contaminants virtual screening allows putative binding mode identification before experimental testing. In this study, molecular docking was applied to the Methoprene-tolerant receptor (Met), the juvenile hormone receptor in insects and crustaceans. Met is naturally activated by methylfarnesoate in crustaceans, but can be disrupted by pesticides like fenoxycarb, severely impacting the reproduction of non-target species like *Daphnia magna*. Daphnids are key primary consumers in aquatic ecosystems, thus understanding Met activation and identifying interacting compounds is essential for ecotoxicological risk assessment. A previous study tested the ability of 28 environmental chemicals to activate Met *in vitro*. Based on these results, we evaluated the tested compounds' interactions with Met using molecular docking, with the aim of validating an approach for screening a large number of environmental contaminants to identify potential endocrine disruptors. We also tested our approach on furosemide (FUR), a pharmaceutical contaminant and two of its metabolites, pyridinium of furosemide (PYR) and saluamine (SAL) which presented significant toxicity at environmentally relevant concentrations (ng/L).

The 3D structure of *D. magna* PAS-B domain (ligand binding site of Met) has been modelled using AlphaFold DB and the screening box was designed based on previous studies. Flexible docking was run on the 28 compounds studied *in vitro*, FUR, PYR and SAL. Docking results were assessed by 15-day reproduction assays on *D. curvirostris* with FUR, PYR, SAL and mixtures.

Docking results indicate that *in vitro* active compounds have low binding energies, consistent with favorable interactions with Met. However, some inactive compounds showed similarly low energies. Most of these false positives contain chloride atoms, which may form halogen bonds with Met *in silico* only considering that none of the active compounds contain chlorides. FUR and PYR which also contain chlorides, displayed low binding energies, suggesting potential interactions with Met. However, like SAL, they showed little to no impact on *D.*

curvirostris reproduction in vivo. Assays on *D. magna* will soon be performed to confirm these results. Additional docking and interaction analysis with Met are ongoing to improve mechanistic understanding and enable broader-scale applications.

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1.07.T-03 Genome Editing *Daphnia* as a Tool for Understanding Endocrine Disruption

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Juvenile hormone (JH) regulates essential biological processes in arthropods, including reproduction, molting, and sexual differentiation. However, in non-insect crustaceans such as *Daphnia magna*, the JH-producing organ and regulatory mechanisms remain poorly characterized, making it difficult to assess how environmental contaminants disrupt JH-mediated physiology. The objective of this study is to establish genome-edited *D. magna* lines capable of visualizing JH biosynthesis in vivo, and to utilize them for evaluating endocrine disruption caused by juvenile hormone analogs (JHAs).

To identify the JH-producing organ, we generated a TALEN-based intron knock-in strain in which Achilles fluorescent protein is bicistronically expressed from the juvenile hormone acid o-methyltransferase (JHAMT) locus, a key enzyme in the sesquiterpenoid biosynthesis pathway. Reporter expression was confirmed in F1 offspring. Fluorescence localization revealed a previously unidentified bilateral JH-producing organ located posterior to the brain in juveniles, suggesting an endocrine secretion route that mediates multiple physiological functions.

To evaluate endocrine disruption, we examined fenoxycarb toxicity using time-course exposure and transgenic imaging. A vasa:H2B-GFP strain enabled direct visualization of ovarian cell integrity. Fenoxycarb exposure significantly reduced fecundity and induced apoptotic nuclear abnormalities in developing oocytes, demonstrating that JHA-induced reproductive impairment is mediated through oocyte cell death.

In conclusion, genome-edited *D. magna* strains enable real-time visualization of endogenous JH signaling and provide mechanistic insight into endocrine disruption by JHAs. These tools strengthen the utility of *Daphnia* as a sensitive and biologically relevant model for environmental risk assessment of endocrine-disrupting chemicals in aquatic ecosystems.

1.07.T-04 Understanding the Impacts of Exposure to Ten Pesticides and Their Mixtures in *Daphnia pulex*: a Transcriptomics Approach

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Freshwater systems are highly diverse environments supporting an important component of the world's biodiversity but are highly vulnerable to environmental stress, including that associated to chemical exposures. In river catchments affected by farming, some of the most common contaminants are pesticides. Given the complex nature of the chemical contamination present in these environments, it is challenging to assess to what extent organisms in these ecosystems are affected by chemical exposures. As a result, for many of the chemicals present in freshwater systems, their impact on freshwater invertebrates is poorly understood. We aimed to develop and apply a transcriptomics approach to determine the mechanisms of toxicity of 10 common pesticides, alone and as part of mixtures, to better understand their impacts on the health of freshwater invertebrates. To do so, we used the *Daphnia pulex* as a model system due to its well characterised genome and history of use as an ecotoxicology model. We combined exposure experiments with transcriptomics (RNA-Seq in a NovaSeq S4 Illumina platform) over time to determine the adverse effects of each individual chemical alone and as part of binary and complex mixtures. Our data revealed specific mechanisms of action for each chemical with commonalities also encountered, in particular for molecular pathways associated with response to stress and recovery. Gene Set Enrichment Analysis for the mixtures showed some degree of concordance between the molecular pathways affected by individual chemicals and the mixtures showing that the effects of chemicals alone can predict some, but not all, the outcomes of complex mixtures. We are now expanding our analysis to include weighted correlation network analysis in order to uncover global trends across all the dataset.

1.07.T-05 Different pasts, different responses: How Daphnia react to cyanobacteria exposure?

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Freshwater ecosystems are increasingly threatened by cyanobacteria, which compromise water quality and affect aquatic organisms. *Daphnia*, a key genus in freshwater food webs, often coexists with cyanobacteria, facing physiological stress that can drive adaptation or tolerance. This study aimed to integrate the morphological and genetic identification of *Daphnia* and cyanobacteria from Portuguese reservoirs and evaluate how different field *Daphnia* populations respond to cyanobacteria through feeding performance and biomarker analyses. Two Portuguese reservoirs were selected: Rabagão (bloom-free) and Aguieira (bloom-prone) to collect water samples and *Daphnia* individuals. Cyanobacteria and *Daphnia* species were isolated and taxonomically and genetically identified. Feeding inhibition assays were conducted with *Daphnia magna* and field *Daphnia* spp. exposed to cyanobacteria cell concentrations (cells/mL): 7.5×10^7 (medium risk), 1.5×10^7 (high risk), 3.0×10^7 (high risk), and 1.2×10^7 (very high risk), according to World Health Organization guidelines. Additionally, biomarkers related to oxidative stress, neurotoxicity, and energy metabolism were evaluated. Three *Daphnia* species were identified: *D. longispina* and *D. middendorffiana* associated to Rabagão and *D. galeata* and *D. middendorffiana* to Aguieira. *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* were identified only in Aguieira, while Rabagão showed no bloom-forming species. *D. magna*, *D. middendorffiana* (from Rabagão) and *D. galeata* (from Aguieira) exhibited feeding inhibition under cyanobacterial exposure, though feeding rates increased with cyanobacteria concentration. Antioxidant enzyme activity (e.g. catalase) was enhanced, as well as oxidative damage, acetylcholinesterase inhibition, and altered energy metabolism after *Daphnia* spp. was exposed to cyanobacteria. Populations from the bloom-prone reservoir (*D. galeata*) displayed stronger physiological adjustment and distinct biomarker profiles, suggesting higher cyanobacteria tolerance. These findings highlight that cyanobacteria can impair *Daphnia* even at concentrations posing human health risks, and that exposure history influences tolerance and physiological responses to bloom conditions.

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1.07.P Daphnia- Advances, Problems, & Historic, Novel and Interdisciplinary Approaches

1.07.P-Mo017 Assessing Sublethal Effects of Nanomaterial-Enhanced Magnesium Oxychloride Cement (MOC) Composites on *Daphnia magna* Through Feeding and Biomarker Responses

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Magnesium oxychloride cement (MOC) is considered a more environmentally friendly alternative to conventional cement. Unfortunately, some of its properties limit its potential applications. Therefore, the possibility of incorporating various additives to improve its properties is being investigated (e.g. reducing hydrophilicity and increasing strength). One option for these additives are nanomaterials, such as multi-walled carbon nanotubes (MWCNT), oxidised multi-walled carbon nanotubes (MWCNT-ox), graphene oxide (GO) or graphene (G). Although these compounds can improve properties, they may have a toxic effect on organisms relevant to the environment, such as the microcrustacean *Daphnia magna*. This study investigated the impact of these materials on the feeding behaviour of *D. magna* after 24 hours exposure at concentrations of 10, 50 and 100 mg/L. Biomarkers associated with cholinergic neurotransmission (acetylcholinesterase – AChE activity), oxidative stress (glutathione-S-transferase – GSTs activity) and lipid peroxidation (thiobarbituric acid reactive substances – TBARS levels) were quantified to evaluate the effects on a sub-individual level. In terms of feeding, a significant increase was observed, especially at the highest tested concentrations after exposure to MOC, MOC-MWCNT-ox and MOC-GO, whereas a decrease in feeding rates was recorded following MOC-G exposure. Regarding neurotransmission, only 50 and 100 mg/L of MOC-GO cause a significant increase in

AChE activity. All the tested compounds caused significant differences in GSTs activity, suggesting an activated enzymatic response after exposure to these stressors. Although these significant responses observed, the results indicate that enzymatic activity was sufficient to prevent lipid peroxidation, as no oxidative damage was detected. These findings demonstrated that while the incorporation of nanomaterials may enhance the physicochemical properties of MOC, these additives can also amplify or modify its toxicity toward aquatic organisms. Given the ecological importance of *D. magna* and its role in freshwater ecosystems, these results highlight the need for careful evaluation of MOC-based materials to prevent potential environmental impacts across trophic levels.

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1.07.P-Mo018 Behavioral Analysis of Freshwater Invertebrates – A Deep-Learning Approach for 3D Movement Analysis

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Global change significantly impacts freshwater ecosystems, contributing to biodiversity loss and ecosystem instability. Disregarding the type of stressor, one of the first adaptations of organisms to changes in the surrounding environment is behavioral. Analysing these behavioral changes still remains challenging, but in the recent years the multitude of computational-assisted methods have become increasingly important. However these analytical approaches are often semi-automatic, thus subjective and prone to errors, requiring a time consuming manual correction. Most of the approaches are only focusing on the organism used in the specific laboratory, making it hard to transfer the software to other labs. Additionally only few of them are designed to analyse movement of smaller organisms such as invertebrates and even less are able to analyse movements in the three dimensional space. However this is crucial for the analysis of organisms living in aquatic systems. Here we present a concept for novel approach to analyse sublethal effects of different stressors on the behavior of different freshwater invertebrates, mostly focusing on *Daphnia* spec. Within this workflow, a deep learning-based 3D analysis software is set to be developed, in order to be able to objectively assess even the smallest differences. Therefore general parameters, such as velocity, depth selection as well as neighbor distances will be recorded. Additionally organism specific movement parameters will be analysed. Through the implementation of a fully automatic software, results will be deterministic, thus reproducible and independent of the experimenters experience. The software will mostly be trialed for multiple stressor experiments analysing the effects of psychotropic drugs, such as antidepressants and changes in temperature. However, through the development of an open source software, we will generate a system that is easily applicable to data and experiments from other labs, thus creating a platform of reliable data, with reproducible and comparable results. The software thus offers great potential in studying the effects of different stressors on aquatic organisms, increasing the impact of behavioral freshwater research.

1.07.P-Mo019 Clone-specific responses of *Daphnia magna* to combined warming and contaminant mixtures

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Freshwater ecosystems such as lakes, ponds and pools represent only 1% of the Earth's surface but are dramatically exposed to multiple natural and anthropogenic stressors. Climate warming and chemical contaminants are among the most prominent stressors, increasingly contributing to major biodiversity losses and changes in ecosystem functioning over the last decades.

While the individual effects of warming and contaminants on freshwater ecosystems are relatively well studied, little is known about their combined impacts on aquatic invertebrates, especially when contaminants occur as complex mixtures rather than single compounds. *Daphnia magna*, a model freshwater organism, is commonly used in ecotoxicological studies, but inter-individual variability in response to combined stressors has received limited attention until recently. Moreover, toxicity tests are typically conducted with monoclonal laboratory populations that do not fully reflect the standing genetic variability of natural populations composed of multiple

clones. As a result, standard tests may lead to biased estimates of population-level responses to realistic environmental change.

The present study tests how different clones originating from a single *D. magna* population respond to predicted future warming scenarios and environmentally relevant mixtures of pesticides and pharmaceuticals under laboratory conditions. Individuals were hatched from resting eggs collected in a local pond and maintained in laboratory culture for a year. Chronic toxicity tests were conducted in the laboratory to measure survival, individual growth and reproduction of three clonal lines under two temperature regimes (22°C and 26°C) and four contaminant treatments: control, pesticide mixture, pharmaceutical mixture and the greater mixture of both contaminants. Contaminant concentrations were chosen to reflect environmentally relevant 'worst-case' levels commonly detected in local aquatic environments. Each treatment was replicated 15 times. The measures of individual-level responses were then used to estimate long-term effects of the stressors on the population growth rate of each clonal line.

This work aims to provide insights into inter-individual variability in response to multiple stressors and improve our understanding of how freshwater invertebrate populations may respond to the combined pressures of climate change and chemical pollution.

1.07.P-Mo020 Effects of the antiparasitic veterinary drug ivermectin on *Daphnia magna* including transcriptomic fingerprinting

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Antiparasitic agents are used as veterinary pharmaceuticals e.g. in livestock, and may be released into the environment with potential toxicity to non-target organisms. These active substances can disrupt ecological balance, affect environmental integrity with potential consequences for biodiversity. Ivermectin is an anthelmintic, belonging to the avermectin substance group and is mainly used to treat several types of parasitic infections of livestock and pets. Ivermectin may enter the environment directly through faecal excretion of livestock grazing on fields or using manure as fertilizer. Run-off caused by rainfall events can then lead to the entry into nearby small water bodies. Ivermectin blocks the neurotransmitter γ -aminobutyric acid in invertebrates, thereby inducing paralysis and death of parasites as well as in non-target organisms. In several terrestrial and aquatic invertebrates high ecotoxic potential with effect levels down to the ng/L range was already shown for ivermectin. Even though there are indications of adverse effects on several invertebrates, there is a lack of reliable studies on the effect of ivermectin on the model organism *Daphnia magna* with regard to chronic effects. Therefore, we performed a GLP study to investigate the effects of ivermectin on the reproduction of *D. magna* following OECD test guideline 211. Based on pre-tests targeting acute toxicity (immobilization), we performed the test in a concentration range of 1 - 15 ng/L. In this study, the endpoints immobility, growth, and reproduction, as well as day of first reproduction were evaluated. To confirm the exposure concentrations in this low concentration range, a UHPLC-MS/MS method with a limit of quantification (LOQ) of 0.3 ng/L was developed and validated following SANTE 2020/12830 guideline. In addition, a second short term exposure study (48 h) will be performed to investigate transcriptomic changes hinting at potential further adverse effects in juvenile daphnids. This test aims at identifying potential additional toxic modes of action (MoA) of ivermectin in invertebrates. Furthermore, it is intended to assess the transcriptomic point of departure (tPOD) for comparison of effect thresholds to the regulatory acute and chronic tests.

Overall, these investigations help to understand the toxicity of ivermectin to invertebrates, as well as toxic MoA, and contribute to a better understanding of its potential ecological impacts.

1.07.P-Mo021 Growth Measurements as a Phenotypic Endpoint for the Assessment of the Impact of Pollutants on Water Fleas – An Online and AI Assisted Approach

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The rise in anthropogenic pollution is exerting profound negative effects on both environmental systems and human health, having become the leading cause of death worldwide, surpassing all major diseases. The urgency of tackling these environmental challenges and gaining mechanistic information about anthropogenic contaminants commonly found in the environment is leading to the widespread use of Novel Approach

Methodologies, towards more predictive and holistic methods in chemical risk assessment. In this context, the cladoceran *Daphnia magna* has been widely employed as a bioindicator species in effect-based monitoring approaches, largely due to its ecological importance within freshwater food chains. Phenotypic endpoints have been proven to be sensitive, effective and inexpensive tools to assess pollution levels and the effects of xenobiotic compounds present in freshwater at low but environmentally relevant and biologically active concentrations. Size and growth measurements are important physiological characteristics and represent good sublethal toxicity markers in ecotoxicological studies. The research for an effective standardisable method is leading towards the miniaturisation and automation of phenotypic assays. This study focuses on the development of a growth assay using size measurements as an approach to monitor the impact of pollutants on daphnids. The method was optimised using a low volume and higher volume approach and with different amounts of algae as food to identify the best working conditions. The final method was developed in a small-case volume and was applied to test cigarette extracts. Images of the animals were taken using different instruments (stereoscope, magnifying lenses for phone camera), to assess resolution and aim for improved method portability, and the size measured using a combination of manual (Image J) and automated software (Image Measurement, QuPath). In this context, Artificial Intelligence has been proven to be a useful tool in toxicology due to its predictive and automated capabilities. This study implemented growth assays by developing an AI script for automated size measurements, to improve the efficiency of phenotypic assays in daphnids and their role as non-invasive and rapid screening tools for pollution assessment and early detection systems.

1.07.P-Mo022 Impact of Age and Nutritional Status on Effects in *Daphnia* Acute Studies

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Keywords: *Daphnia magna*, acute test, age, nutritional status, multiple stressors, toxicity endpoints

Acute toxicity tests with *Daphnia magna* (OECD test guideline 202) are currently conducted with juvenile daphnids which should be no more than 24 h old. This means that the age of the animals at the start of the test can differ between 0 and 24 h.

Further, according to the guideline, the daphnids are not fed during the 48-h exposure period, but no information was provided in the guideline regarding the feeding status of the daphnids prior to the start of the test. However, in the light of more precise and comparable results it needs to be questioned whether the age of the test organisms and their nutritional status at test start can have an impact on the sensitivity and thus the resulting EC50 values.

To evaluate possible impacts of the age of the daphnids and their nutritional status at test start acute toxicity tests with *Daphnia magna* (OECD 202) were conducted with three narrowly defined age classes (0 – 4 h, 4 – 8 h and 8 – 12 h). Tests included daphnids that did not come into contact with food prior to exposure and such daphnids which had access to food prior to exposure. All age groups with both nutritional statuses were tested with lambda-cyhalothrin and prothioconazole. EC50 values of the tests runs with the three different age groups in the different nutritional status were compared to evaluate possible differences and thereby impacts of age and nutritional status on results of toxicity tests with *Daphnia magna*.

1.07.P-Mo023 Improving micro-/nanoplastics (MNPs) ecotoxicity assessment: A harmonised ecotoxicity testing approach with *Daphnia magna*

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Micro- and nanoplastics (MNPs) are emerging contaminants of global concern, yet their ecological risks remain poorly defined due to limitations in current toxicity testing. Standard assays with *Daphnia magna*, a keystone grazer and regulatory model species, typically employ pristine, monodisperse polystyrene MNPs and focus on endpoints such as immobilisation or reproduction. This simplified approach neglects the freshwater cladoceran *Daphnia magna*'s ecological function, fails to capture the diversity and weathering of environmental plastics, and generates fragmented datasets that hinder meta-analysis and robust risk evaluation. To address these shortcomings, a tiered framework for MNP ecotoxicological assessment using *D. magna* that integrates ecological relevance, mechanistic insight, and systematic data harmonization is proposed. The framework progresses from acute endpoints (mobility, behaviour) to chronic assays (growth, reproduction, population dynamics), multigenerational studies (offspring quality, ephippial investment, sex ratio, transgenerational resilience), and mechanistic investigations employing omics and epigenetics. A central principle across all tiers

is environmental realism, operationalised through the inclusion of MNP characterization, heterogeneous and weathered MNPs, diverse polymer chemistries, MNP physicochemical heterogeneity, and co-occurring environmental stressors. By systematically linking life-history traits with molecular and transgenerational endpoints under realistic exposure scenarios, the framework provides a more comprehensive understanding of MNP effects at both organismal and population levels. Importantly, it establishes protocols for endpoint selection, exposure duration, and test material characterisation, thereby improving comparability and reproducibility across studies. Adoption of this approach will help bridge the gap between laboratory testing and ecological risk assessment, enhancing predictive capacity on plastic pollution risks.

1.07.P-Mo024 Integrated assessment of *Daphnia magna* condition: applications in culture management and ecotoxicological testing

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Daphnia magna is used widely as a standard ecotoxicological indicator organism, and protocols exist for its use in assessing the toxicity of substances under acute and chronic experimental conditions. The objectivity of bioassay results largely depends on the level of “reliability” of the test cultures used. Even though reliability of *Daphnia magna* tests being ensured through standard protocols and quality control measures, such as those defined by the OECD, the biota response may vary due to the overall condition of the cultures used to start the experiments. Hence, the condition/fitness of the cultures plays a relevant role in ecotoxicological assessment. For this purpose, it is useful to assess the reproduction in *Daphnia* cultures, as well as size distribution and individual fitness. Moreover, some multigenerational testing for ecological risk assessment might consider the use of multiple organisms in a single replicate, likely resulting in several individuals of different sizes in each replicate. In this case, it is also useful to apply a method allowing a fast, non-destructive count of *Daphnia* offspring, as well as their size distribution and individual fitness.

The aim of our study is to discuss the feasibility of using an automated, rapid, and non-destructive technology (D-counter) to evaluate *D. magna* condition (reproduction, size and “fitness” of individual organisms) under conditions of high density and a large fraction of juveniles, using light detectors. This is particularly relevant in cultures (to assess the overall condition of the culture) and in multigenerational experiments using multiple individuals per replicate. The D-counter device has already been tested for assessing the number and size of individual daphnids and, hence, the novelty of the present study is the assessment of daphnids “fitness”. Results were obtained applying the automated device D-Counter and using *D. magna* cultures maintained under laboratory conditions, as defined by the OECD protocols. The evaluation of individual “fitness” was obtained as the ratio between the observed opacity of every single organism divided by its estimated length. In *Daphnia*, animal width changes with nutritional status and growth within instar, while length changes only during moulting. Thus, estimation of individual “fitness”, besides evaluating overall culture condition, also allows an integrated characterization of every organism, improving comparability and reliability.

1.07.P-Mo025 Novel New Approach methodologies in *Daphnia magna* to monitor neurological hazardous contaminants: from lab to field, from single to chemical mixtures

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New Approach Methodologies (NAMs) in chemical hazard assessment refer to scientific methods, technologies, or approaches that provide information on chemical safety without relying on traditional animal testing. NAMs thus include existing standardized and novel bioassays with the aquatic crustacean *Daphnia magna*.

Concomitant with the extension of life, there is an increasing concern for neurological disorders. Humans as well as biota are exposed to complex chemical cocktails in the environment, being neurological active contaminants part of them. Thus there is a need to identify neurological hazardous chemical cocktails in the field. Here we present experimental results showing that the *D. magna* can be used as a neurological model to assess chemical mediated neurological defects following exposures during embryonic stages, that *D. magna* is also a good neurological model to study how gut dysbiosis affects the central nervous systems and cognition, and how it is possible to detect chemicals that may promote Parkinson using *D. magna* locomotion kinematics.

Finally, we propose a framework to identify neurological active hazardous chemicals in the field.
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1.07.P-Mo026 Similar Metabolomic Responses of *Daphnia magna* to Insecticides with Different Modes of Action.

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Daphnia magna is used as an aquatic macroinvertebrate model species for laboratory toxicity testing to identify chemical hazards and assess chemical risks. The Adverse Outcome Pathway framework enables the understanding of how a contaminant's mode of action affects an organism and where these effects occur. This framework is based on the principle that if a contaminant results in a similar molecular initiating event, a cascade of key events downstream will follow a similar pathway. It can be expected that a similar pathway and adverse outcome can then be expected. These outcomes can include respiration, swimming behaviour, and reproduction, just to name a few. This study aimed to investigate the effect of two insecticides with different modes of action on the metabolic profile of *Daphnia magna*. This study applied an untargeted metabolomics-based approach to identify metabolites and metabolic pathways affected after exposure to imidacloprid and deltamethrin. Daphnids were exposed for 48 hours to sublethal concentrations of 25.5 mg/L imidacloprid and 0.4 µg/L deltamethrin. These concentrations are based on 30% of the LC50 for each insecticide. Metabolomic profiling revealed disruptions in the amino acid, carbohydrate, and lipid metabolism, where both insecticides disrupted overlapping pathways that are energy related and oxidative stress. Deltamethrin caused a much stronger metabolic impact, with more metabolites significantly affected in specific pathways. While imidacloprid mainly altered carbohydrate, sugar, and general energy metabolism, deltamethrin severely disrupted amino acid, nitrogen, glutathione, and nucleotide pathways. This indicates a more intense oxidative and cellular stress response. Overall, both pesticides target similar metabolic systems regardless of the mode of action which will result in similar adverse outcomes. Nevertheless, deltamethrin induces more severe pathway-level disturbances at significantly lower concentrations.

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1.07.P-Mo027 Susceptibility of *Daphnia magna* to immune-stimulating and immune-suppressing effects of pharmaceuticals

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Within the world of human-use pharmaceuticals, immune-stimulants and immune-suppressants are used to support the human immune system, e.g. to fight off viruses, or to suppress immune reactions that would e.g. lead to the rejection of transplanted organs. Environmental organisms exposed to pharmaceuticals targeting the immune system may not only show signs of general toxicity, but also could be affected by specific immunotoxic effects. The innate immune system specifically is evolutionary highly conserved, and represents the key defence mechanisms of invertebrate organisms lacking an adaptive immune system. While the current environmental risk assessment for human-use pharmaceuticals requires a targeted analysis for pharmaceuticals with specific modes of action, immune-related specific effects are not explicitly mentioned. In addition, there are no established test guidelines for investigating such potential effects in invertebrates. In this presentation, we demonstrate the use of an invertebrate host-parasite model, involving *Daphnia magna* as host, for investigating the influence of an immune-stimulant as well as an immune-suppressant on the outcome of the host's battle with its bacterial endoparasite *Pasteuria ramosa*. We observed that both pharmaceuticals influenced the host-parasite interaction in a way that is in agreement with the intended use of the pharmaceuticals in human patients. These results demonstrate that *D. magna* is a suitable model for studying immunotoxicity in invertebrates and, secondly, that pharmaceuticals targeting components of the innate immune system can have specific effects in exposed invertebrate organisms.

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1.07.P-Mo028 The impact of tobacco extracted compounds on daphnids

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There is a growing global recognition of the devastating effects of pollution, which remains one of the most pressing environmental challenges of our time. Among its many sources, tobacco pollution represents an overlooked contributor with serious ecological consequences. While the health impacts of smoking are widely acknowledged, the environmental burden of tobacco production, consumption, and waste is equally alarming. Discarded cigarette butts, which constitute one of the most common forms of litter worldwide, can persist in the environment for up to fifteen years. During this time, they release a complex mixture of toxic chemicals including nicotine, heavy metals, and polycyclic aromatic hydrocarbons into surrounding soils and water bodies. This leaching process contaminates aquatic ecosystems, threatens wildlife, and contributes to the broader issue of chemical pollution. Addressing tobacco pollution, therefore, requires not only public health interventions but also improved waste management and policy measures to mitigate its long-term environmental impact. Current pollution assessments primarily focus on the detection of chemicals in the environment to estimate water quality; however, these methods are often descriptive and insufficient for understanding ecological impacts. This limitation underscores the need for effect-based methods as more sensitive tools for risk assessment. Daphnids are key species in molecular ecotoxicology and serve as effective indicators of environmental health.

In this study, we employed a combination of phenotypic and holistic approaches to investigate the impact of extracts from tobacco on these aquatic organisms. Integrating assessments of toxicity, growth, survival, feeding behaviour, and key enzymatic activities revealed distinct molecular and physiological responses, which serve as “molecular fingerprints” of exposure to tobacco-derived contaminants. This comprehensive approach highlights the potential of the water flea as a sensitive bioindicator to assess ecological risks from tobacco pollutants, acting as an equivalent to a “canary in the coal mine” for freshwater ecosystems.

Disclaimer/Disclosure:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this abstract.

1.07.P-Mo029 The short-term Juvenile Hormone Activity Screening Assay (JHASA): intra-laboratory findings and statistical issues.

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Juvenile hormones (JHs) play crucial roles in the developmental and reproductive processes of *Daphnia magna*. Several studies have demonstrated that JHs and their analogs, such as the insect growth regulators pyriproxyfen and diofenolan, are involved in sex determination. Exposure to these compounds induces the production of male neonates in subsequent broods of *D. magna*. Consequently, the production of male offspring is a relevant endpoint for detecting endocrine-disrupting chemicals (EDCs) with JH activity. The OECD Test Guideline 253, “Short-term Juvenile Hormone Activity Screening Assay in *Daphnia magna*”, was developed to screen substances for short-term JH activity and detect their endocrine-disrupting potential. In this assay, *Daphnia* aged 10–17 days are exposed until their second reproduction, and the sex of the offspring from this brood is recorded. If male offspring are produced in treated groups and none in controls, the substance is considered “active” without the need for statistical analysis.

In this study, two molecules known to act as juvenile hormone analogs in *D. magna*, pyriproxyfen and diofenolan, were tested. Preliminary OECD TG 202 assays were conducted to determine the EC₅₀ values for each substance (0.283 mg/L for pyriproxyfen and 0.328 mg/L for diofenolan). To avoid acute effects, half the EC₅₀ was used as the maximum concentration in the definitive assay. Three concentrations were tested for pyriproxyfen (30, 70, and 140 µg/L) and five for diofenolan (10, 20, 41, 82, and 172 µg/L).

In the definitive tests, no males were observed in the controls, whereas a high proportion of males appeared at low concentrations of both substances. The sex ratio effect was evident from the lowest dose tested, and reproduction decreased with increasing concentration.

Beyond the results of our study, we identified some issues with the statistical approach recommended in the TG when a dose-response curve is based on more than three treatment levels. These issues relate to the recommended procedure for calculating ratios, the use of arcsin transformation regardless of sample size, and the explicit advice against using the Cochran-Armitage test, despite this being a powerful method for analyzing quantal data, including extrabinomial variance.

Our findings confirm the sensitivity of *D. magna* to JH analogs and highlight the need for refinement of OECD 253's statistical recommendations to ensure correct data interpretation in endocrine disruption screening.

1.07.P-Mo030 To What Extent Can the Fungicide Azoxystrobin Affect the Uptake and Elimination of Copper in *Daphnia magna*?

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Copper (Cu) is essential for a well-functioning metabolism in almost all living organisms. However, like most essential metals, Cu can cause toxicity when internal concentrations exceed essential needs. To a certain extent, organisms can regulate their internal Cu concentrations to ensure homeostasis and avoid toxicity. In aquatic systems, metals often occur as mixtures with other chemical micropollutants, both organic and inorganic. The uptake and elimination processes of metals have been extensively studied in many aquatic organism's but less is known about how these processes can be affected by the presence of organic substances.

We investigated the extent to which the presence of the fungicide azoxystrobin affects the uptake and elimination of Cu in *D. magna*. Azoxystrobin is a mitochondrial respiration inhibitor that affects the organism's energy metabolism. Based on its MoA, we hypothesised that azoxystrobin may cause an increased uptake and decreased elimination of Cu in *D. magna*. The study employed a stable isotope (⁶⁵Cu) approach to determine the uptake rate over a 72-h period and the elimination rate after 24 h of elimination. In the uptake phase, *Daphnia* were sampled every 2h for 12h, after 24h, 48h and 72h and internal ⁶⁵Cu concentrations were measured. In the elimination experiment, *Daphnia* pre-exposed for 48h to ⁶⁵Cu were transferred to clean medium and sampled every 2h for 10h and after 24 h. Per time point, three replicates of 10 individuals (5-6 days old) were sampled. Based on earlier chronic reproduction tests, the effect concentrations (EC) of copper of 5% and 25% effect were used in this study. The tested treatments were ⁶⁵Cu alone, azoxystrobin alone, a binary mixture of ⁶⁵Cu-azoxystrobin and a control treatment. A two-compartment model was applied to derive uptake and elimination rate constants. The differences between uptake and elimination rate constants of Cu alone and in the presence of azoxystrobin were interpreted. Preliminary results indicate that the presence of azoxystrobin increased ⁶⁵Cu uptake rates. These findings allow a deeper understanding of the interactions metal-organic mixtures have on organisms like *D. magna*. In the future these results will be used to optimize toxicokinetic models to enhance the predictions of internal metal concentrations in mixture situations with organic substances.

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1.07.P-Mo031 Water fleas as “canaries in the coal mine” for the detection of cigarette-derived pollution in the environment

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Smoking is a leading global habit with implications for human health and the environment. While the adverse health effects of smoking have been extensively studied, the environmental impact of cigarette-derived pollutants remains underexplored. The waste produced from discarded cigarette filters takes up to fifteen years to decompose and leach out toxic chemicals in aquatic environments. Various analytical techniques employed in water monitoring identify contaminants, however, these methods are inadequate to provide early predictions and capture the underlying mechanisms for the observed effects in the ecosystem. This limitation highlights the need for comprehensive approaches in pollution assessment. Consequently, there is growing support from effect-based methods that assess the responses of key species, such as daphnids to pollutants. Daphnids are critical freshwater organisms that serve as valuable indicators of ecosystem health. In the context of New Approach Methodologies, daphnids provide significant advantages due to their alignment with the principles of the 3Rs (Reduce, Refine, Replace) and their conserved toxicity responses to more complex organisms, in phototoxicological studies. Our research focuses on the impact of smoked cigarette and e-cigarette filters on the

physiology and metabolism of neonates and four generations of *Daphnia magna*, followed by analysis of filter extracts to assess their chemical composition. By integrating phenotypic endpoints, such as growth, feeding and reproduction, with metabolic fingerprints, we aim to provide deeper mechanistic insights into the actions of these pollutants. Ultimately, identifying metabolic disruptions can yield more sensitive assessment metrics, allowing us to capture the mechanisms of action more accurately. This enables timely predictions of pollution hotspots, facilitating proactive management strategies to mitigate environmental damage before it becomes irreversible.

1.08 Democratization of Big Data: Live Demonstrations of Computational Platforms and Data Resources to Advance Chemical Safety Assessment

1.08.T-01 Navigating Chemical Spaces with COMPASS: Standardized Visualization of Chemical Data Sets

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Chemical safety assessments are often limited by missing data needed to describe environmental fate, exposure, and effects on human and ecosystem health. Data-driven prediction methods using machine learning and artificial intelligence offer promising ways to fill these data gaps by estimating chemical properties directly from molecular structures. However, because these models rely on existing data, their reliability is restricted to chemicals similar to those included in their training sets. Currently, there is no standardized reference to compare the applicability of different models across the wide range of chemicals in use. To address this, we developed COMPASS (COMParative chemical Space Systems), a user-friendly application that visualizes reference chemical spaces for marketed chemicals and four application-oriented domains: pharmaceuticals, pesticides, PFAS, and natural products. These reference chemical spaces were created using well-curated reference lists and visualized through t-distributed stochastic neighbor embedding (t-SNE), which maps the high-dimensional structure of each domain into an intuitive two-dimensional space. The developed interactive tool enables users to explore chemical diversity and overlap across data sets. Model developers can use it to assess how representative their training data are and identify chemical domains where prediction reliability may be limited. Experimental researchers can use it to prioritize data generation for underrepresented compounds, while tool users can compare the applicability of different models within a given chemical space. By supporting more transparent and harmonized applicability assessments, the tool can strengthen confidence in chemical predictions and support iterative improvements in both data coverage and model representativeness across diverse chemical domains.

1.08.T-02 AMIRA: Animal-free Mechanistic Inference for Risk Assessment

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Environmental Risk Assessment (ERA) of chemicals is essential for regulatory approval and environmental protection. This traditionally requires testing across taxonomic groups of algae, crustaceans, and fish to determine the most sensitive species. With regulatory bodies drafting roadmaps to phase out animal testing in line with the 3Rs principle, it has become essential to learn from existing *in vivo* vertebrate data to inform the development of predictive models and support future risk assessments. Previously, we have shown that vertebrates exhibit higher sensitivity than invertebrates and algae for only 10% of chemicals in acute toxicity studies and 16% in chronic toxicity studies, indicating that data from algae and invertebrates may often be sufficient to establish environmental quality standards. Existing ecotoxicity databases do not offer a framework for systematically comparing inter-species and species-sensitivity patterns across chemical classes and taxa while also integrating mechanistic information.

To address this gap, we developed an interactive web application, AMIRA, that integrates ecotoxicity data across the entire chemical landscape, combining the U.S. EPA ECOTOX Knowledgebase (>10,000 chemicals, 6,395 species) with a curated dataset of 205 pharmaceuticals. Users interested in inter-species sensitivity can access harmonised acute and chronic toxicity datasets for, respectively, 631 and 674 chemicals. These represent chemicals with data from vertebrates and algae or invertebrates, enabling meaningful cross-taxon comparisons. AMIRA also enables users to identify chemicals that are more, less, or similarly sensitive within selected taxonomic groups by comparing statistical summaries and applying uncertainty factors. This approach facilitates

the identification and prediction of chemicals for which testing in non-vertebrates could replace fish studies without compromising environmental protection goals.

By integrating inter-taxa sensitivity patterns with pathway-based insights, AMIRA will support the mechanistic understanding of why species may be more sensitive to given chemical classes, enabling the prioritization of chemicals based on regulatory-relevant mechanisms of action and enable predictive modelling of untested chemicals rooted in biology. Interactive demonstrations at the session will showcase the potential of curated, interoperable datasets to accelerate ethical, efficient, and mechanistically informed chemical safety assessments.

1.08.T-03 PULSE: A Pilot UVCB (Substances of Unknown and Variable Composition, Complex Reaction Products or Biological Materials) Library for Substance Prioritization

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Substances of unknown or variable composition, complex reaction products, and biological materials (UVCBs) account for a significant portion of chemicals registered globally and pose challenges for risk evaluation. To address these issues, the UVCB committee of the Health and Environmental Sciences Institute (HESI Global) has been developing a tiered, exposure-based framework to support UVCB prioritization. The initial tier (Tier 0) emphasizes basic characterization to identify potential exposure concerns, but this step depends heavily on the availability of fundamental compositional, exposure, and other data—information that is often sparse or inconsistent in public sources.

To help fill this gap, the HESI UVCB Committee developed PULSE, a Pilot UVCB Library for Screening and Evaluation. PULSE compiles Tier 0 information for 109 UVCBs selected from the Canadian Domestic Substances List and the OECD PFAS inventory. Data were collected across nine categories essential for initial assessment, including identifiers, structural descriptors, whole-substance ecotoxicity, constituent information, physicochemical properties, environmental fate, manufacturing and use data, registration status, and analytical metadata. Publicly accessible regulatory dossiers, chemical databases, scientific literature, and industry documents served as data sources. Since UVCB data are often incomplete or conflicting, it was very important that each source was assigned a qualitative reliability score to ensure transparent interpretation.

Data completeness varied substantially. Identification information was generally robust, while structural, physicochemical, and fate data were frequently partial or presented as ranges. Constituent data were limited for many substances, reflecting broader challenges such as inconsistent CAS mappings, limited public reporting, and the incompatibility of complex mixtures with single-compound database structures.

This pilot effort demonstrates the feasibility and value of a structured Tier 0 UVCB database. PULSE supports rapid screening, prioritization, and read-across within the developing UVCB framework and highlights areas where improved reporting and collaboration are needed to fully realize the database's potential.

Disclaimer/Disclosure:

The views expressed in this poster are those of the authors and do not necessarily reflect the views or policies of their respective institutions.

1.08.T-04 envPath: Transformer-based Biotransformation Pathway Prediction and Open APIs for Regulatory Workflows

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Experimental pathway elucidation relies on long-term laboratory studies that are resource-intensive, time-consuming, and costly to scale. As a result, risk assessors and regulators often lack complete information on transformation processes, limiting their ability to identify persistent or hazardous transformation products.

enviPath addresses these challenges by combining pathway prediction models with an open, expert-reviewed database. The new transformer-based model is trained on curated biotransformation data and complements rule-based systems by proposing plausible transformations and pathway structures, including for chemistries with limited rule coverage. Alongside prediction, envPath serves as registry for curated transformation data—such as recent PFAS-focused datasets; reducing fragmentation and enabling users to search, retrieve, and compare deposited reactions with model-generated pathways. A redesigned web interface supports intuitive exploration of pathways and transformation records, while a documented REST API enables integration into automated screening, prioritization, and modelling workflows.

In this live demonstration, participants will walk through representative assessment tasks using both the interface and API: predicting pathways for candidate substances, identifying potentially persistent transformation products, retrieving relevant deposited reactions, and exporting results for downstream analysis or dossier preparation. Attendees will also have the opportunity to discuss practical use cases and future feature needs directly with the developers.

1.08.P Democratization of Big Data: Live Demonstrations of Computational Platforms and Data Resources to Advance Chemical Safety Assessment

1.08.P-Mo032 High-Throughput Screening and Structure–Activity Relationship Analysis of ER α -Targeted Toxicity for Emerging Pollutants

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The estrogen receptor alpha (ER α) plays a critical role in mediating endocrine-disrupting effects of emerging pollutants, which puts higher demands on the classification and categorization of chemicals may have strong affinity based on computational prediction. In this study, we combined virtual screening and quantitative structure–activity relationship (QSAR) modeling to identify and predict the high ER α -affinity emerging contaminants. Firstly, a curated dataset of more than 150,000 emerging contaminants from the EPA and PubChem data sets were screened using molecular docking to determine binding affinities with ER α . Compounds with favorable binding energies (lower than -5.0 kcal/mol) were further analyzed for ER α -compound interaction. Here, machine learning-based QSAR models were then developed to capture the ER α -compound interaction using molecular parameters. Through expertise assistance and rigorous modeling methodology, an accurate and robust Extreme Gradient Boosting (XGBoost) model was established and key molecular structures influencing the binding energy were identified through SHAP analysis, including phenolic, stilbene, and halogenated aromatic motifs. The combined workflow enabled the identification the chemicals with potential of high binding energy with ER α . The predictions demonstrated the good generalization of ML model which was subsequently validated by in vitro experiments. This integrative approach offers a rapid and mechanistically informed strategy for the high-throughput screening emerging pollutants and prioritizing candidates for experimental assessment.

1.08.P-Mo033 The FACT database: A comprehensive collection of fish acute cell toxicity data.

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One of the barriers towards broader acceptance of New Approach Methods (NAMs) is a lack of confidence into, and understanding of, the capabilities of these assays. Such limitations are often due to the fact that not all relevant data is publicly available and an associated unclear definition of the applicability domain of the assay. The RTgill-W1 cell line acute toxicity assay is an alternative method for fish acute toxicity that has been validated and standardized as OECD Test Guideline 249. Its results have been shown to strongly correlate with fish acute mortality. In order to strengthen the confidence in this method and to characterize the continuously expanding applicability domain of the assay, we created the FACT (Fish Acute Cell Toxicity) database as a collection of currently available viability data from tests using fish cell lines. The database aims to be a comprehensive collection of available cell viability data associated with OECD TG 249. The data is collected through a standardized workflow that includes the assessment and rating of adherence to OECD TG 249. Thus, data is labeled as fully adherent, adherent with some deviations (e.g., different plate formats), or as relevant research data with a clear link to OECD TG 249. To reduce variability in the analysis of the data, concentration-response curves are created and visualized through a coherent and consistent method. Publicly available data is made available through the user interface of the database, which can be searched using the chemical name and a range of chemical identifiers, as well as filtered by experimental properties and OECD compliance level. Data can be exported as Excel or CSV file. Non-public data can be included but are only used internally to continually characterize the applicability domain of the assay. Each data point can be browsed individually through a detailed view. All data can further be explored without computational expertise through the

accompanying FACTexplorer dashboard that provides an overview and summary statistics of the data contained at any point in time. This effort aims to strengthen the confidence in the utility of fish cell lines for chemical hazard assessment through showcasing the breadth of toxicity data and applications.

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1.08.P-Mo034 WATCH: A comprehensive WATER CHEMISTRY dataset for German freshwater systems

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Freshwater ecosystems are among the most biodiverse and valuable habitats on Earth, providing vital ecosystem services to both humans and wildlife. However, they are also among the most threatened ecosystems globally, with biodiversity declines exceeding those of marine and terrestrial systems. European policies, including the Water Framework Directive (WFD) and the Habitats Directive, seek to mitigate these threats by mandating ecological and chemical monitoring of surface waters which is why by 2006 all German federal states established water quality monitoring networks to comply with the WFD, resulting over time in the large-scale generation of georeferenced chemical and ecological data.

Despite this wealth of data, challenges persist due to fragmented data storage, inconsistent parameter terminology, varying measurement protocols originating from Germany's decentralized federal system. Limited accessibility further impedes comprehensive nationwide assessments and integrated freshwater management. To address these issues, we developed the WATCH database (WATER CHEMISTRY dataset for German freshwater systems), compiling data from all German federal states obtained from diverse institutional, governmental, and research organizations. WATCH encompasses data from over 23,000 sites, includes more than 1,500 chemical, physico-chemical, hydrological, and selected biological parameters, covering a timespan from 1929 to 2025, with the core dataset spanning from 2000 to 2020 for all states.

By unifying data from the different sources, WATCH provides immediate access to harmonized data, eliminating time-consuming requests and standardization efforts, enabling large-scale evaluations of pollutant loads, identification of contamination hotspots, assessment of policy effectiveness, and modelling of land use and climate change impacts. It establishes a foundation for evidence-based freshwater management, supporting ecological conservation, restoration, and sustainable governance across Germany.

1.08.P-Mo035 Predicting Environmental Persistence with PEPPER

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Predicting environmental persistence of chemicals is a key aspect in developing Safe-and-Sustainable-by-Design chemicals, in screening substances for potential environmental risk in chemical regulation, and to parametrize exposure models. We have developed two models to predict persistence endpoints from chemical structure: (i) The Wastewater Treatment Plant (WWTP) breakthrough model, a Random Forest regressor trained on over 1000 chemicals with reported WWTP breakthrough data, and (ii) the soil primary biotransformation half-lives model, a Gaussian Process Regressor trained on half-lives observed for ~900 pesticides and pesticide biotransformation products in soil simulation studies. Both models have been fully validated and published, and they are implemented within the PEPPER (Predict Environmental Pollutant PERsistence) framework. Importantly, half-life and breakthrough predictions come with confidence scores to distinguish reliable from less certain predictions.

To make PEPPER models freely and easily accessible to the public, we provide an intuitive user interface at <https://pepper-app.streamlit.app/>. The web application can be used to predict persistence endpoints for single chemicals, or for batches of chemicals. The PEPPER source code is available on GitHub

(<https://github.com/FennerLabs/pepper>), and the PEPPER library can be easily installed via PiPy for a seamless integration into any Python workflow or software. We hope that our prediction tool proves useful to regulators, industry, and academia alike.

1.09.A Fish Model Species in Human and Environmental Toxicology

1.09.A.T-01 Architecture and validation of a scalable, modular, 3Rs compatible workflow to predict acute and chronic effects of plant protection products in fish

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Effective pest management is essential to protect crop yields and food quality under changing climate and agronomic conditions. Chemical plant protection products provide potent, scalable, and cost effective pest management options for farmers worldwide. However, non target organisms can come into contact with these products, which can result in adverse effects. To minimize effects on non target species (including fish), the environmental safety characteristics of candidate molecules must be characterized and, where needed, optimized early during product development. Traditional in vivo fish toxicity tests are time consuming, costly, ethically challenged, and require large quantities of test compounds, making them difficult to scale to the number of candidate molecules under consideration in early stage development.

To address this challenge and advance the next generation of sustainable plant protection products, we have developed a modular, scalable, in vivo free fish screening strategy. By integrating high throughput testing and computational methods into a single workflow, we can now make reliable predictions of acute and chronic effects in fish without the need for vertebrate in vivo studies. In this presentation, we describe the workflow's components and architecture and present results from our initial validation exercise covering 160 molecules with well characterized in vivo effects in fish.

We find that our workflow is well suited to predict both acute and chronic effects in fish for a wide range of plant protection products. In contrast to previous publications, we observe that empirical non vertebrate testing methods tend to underestimate acute effects of plant protection products on fish. For chronic effects, both applied models perform well overall, but tend to underestimate the toxicity of some insecticides.

We discuss the strengths and challenges of developing an in vivo free screening strategy and outline next steps to expand the workflow's capabilities. In summary, our approach enables the selection, optimization, and de-risking of candidate molecules without reliance on in vivo fish studies, aligning with Bayer's commitment to the 3Rs principles.

Disclaimer/Disclosure:

All authors are employed by Bayer Crop Science, a manufacturer and supplier of plant protection products. The authors are committed to advancing the adoption of New Approach Methodologies (NAMs) in both research and development as well as regulatory contexts, in alignment with Bayer's 3R (Reduce, Refine, Replace) principles.

1.09.A.T-02 Comparative Analysis of Testis and Brain Transcriptomes in Guppies Exposed to Legacy (Perfluorooctanoic Acid) and Emerging (GenX) PFAS

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent contaminants, and growing concerns surround the long-term effects of both legacy compounds, such as perfluorooctanoic acid (PFOA), and newer alternatives such as GenX (trademark name for the ammonium salt of hexafluoropropylene oxide dimer acid). Mechanistic evidence remains limited, particularly for sensitive tissues involved in reproduction and behaviour. This study compared the chronic effects of environmentally relevant levels of PFOA and GenX on the transcriptomes of the testes and brains of adult male guppies (*Poecilia reticulata*). Male and female guppies were exposed for 90 days to PFOA or GenX (1 µg/L). Whole-body concentrations were measured at the end of the experiment, and transcriptomic profiles of the testis and brain were generated using high-throughput RNA sequencing and

differential expression analysis. The aim was to identify tissue-specific molecular pathways responsive to each PFAS and assess whether legacy and emerging compounds trigger similar or distinct mechanisms. PFOA accumulated substantially in fish (bioconcentration factors 3.77–8.30), whereas GenX showed no measurable bioaccumulation. In the testis, GenX altered 22 genes linked to epithelial structure (*krt4*, *cdh17*), osmoregulation, and hormonal signalling (*prlr*), whereas PFOA elicited a modest differential expression in genes. In the brain, GenX triggered a pronounced down-regulation (77 down-regulated genes) of neuroendocrine and stress-related genes (e.g., *prl*, *cga*, *lhb*, *pomc*, *map3k15*), whereas PFOA produced a more heterogeneous effect involving genes associated with synaptic activity, calcium-binding proteins, and lipid metabolism. These findings suggest that GenX may have a greater transcriptomic impact than PFOA under chronic exposure, even in the absence of bioaccumulation. The distinct tissue-specific responses observed across the hypothalamic–pituitary–gonadal axis highlight potential reproductive and behavioral consequences following chronic exposure. This work provides new mechanistic insight into PFAS toxicity and supports improved ecological and human health evaluation.

Disclaimer/Disclosure:

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1.09.A.T-03 The Effect of Thyroid Hormone System Disruption on Neurodevelopment in Zebrafish Early Life Stages

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Thyroid hormone system disruption (THSD) is a major contributor to developmental neurotoxicity (DNT), with adverse outcome pathways linking THSD to impaired neurodevelopment and deficits in cognition, learning and memory in mammals. DNT research has primarily focused on rodent models but zebrafish offer a promising alternative due to rapid brain and central nervous system development within 72 hours post fertilization (hpf) and high conservation of these processes across vertebrates. They also exhibit learning and memory capabilities from early life stages. Additionally, literature shows considerable evidence in the linkage between THSD and DNT in fish. Here, zebrafish embryos were exposed to a known neurotoxicant, chlorpyrifos (CPF), and 6-n-propylthiouracil (PTU), a model THSD compound, from <1 to 120 hpf to assess THSD-related neurobehavioral effects. Learning and memory was evaluated using a Y-maze and custom T-maze assay, both based on spontaneous alternation behavior (SAB, i.e. the animal's tendency to alternate turn direction in consecutive turns). By closing one arm of the T-maze, the animal should choose to explore the other arm on the next trial, which requires proper memory. Data from T-maze and Y-maze, general neurotoxicity tests [spontaneous tail coiling (STC) and touch-evoked escape response (TEER)] and gene transcript analysis was combined to investigate whether THSD impairs learning and memory in zebrafish eleutheroembryos. No preference was observed for the previously free or blocked arm among negative controls for both CPF and PTU, raising the question whether eleutheroembryos exhibit SAB at this age. However, additional control runs with both arms open indicated that 66.67% of eleutheroembryos did exhibit SAB at 120 hpf. Interestingly, both maze assays show that exposed larvae prefer the arm they already visited. CPF exposure led to gene transcript effects on myelin (significant increase in *mbpa* transcript levels and a significant decrease in *sox10*) and pallium (significant decrease in *ascl1a* and *emx1*) related genes. PTU exposure downregulated *mbpa* and *gpr17* - both associated with myelination - and *emx2*. Given that CPF is known to impair learning and memory, and PTU elicits similar behavioral and gene transcript patterns, it is plausible that PTU also disrupts learning and memory. Overall, this work evaluated whether THSD affects the neurobehavioral system and supports the suitability of zebrafish as a model organism for DNT studies.

Disclaimer/Disclosure:

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1.09.A.T-04 High Content Zebrafish Embryo Screening of chemicals and their mixtures

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Understanding how complex chemical mixtures effect organisms remains a major challenge in environmental risk assessment. Mixtures often show combined effects that are difficult to predict. Using the zebrafish embryo model this work addresses whether hydrophobicity-driven baseline toxicity dominates in mixtures of specifically acting chemicals, and whether concentration addition (CA) and/or independent action (IA) models can be also be used to predict sublethal effects. To investigate these questions, we combined specificity assessment with High-Content Zebrafish Embryo Screening using automated imaging and behavioral analysis. Two hundred chemicals were tested across multiple developmental, behavioral, and morphological endpoints, and concentration-response models were generated using a KNIME workflow with embedded R scripts. Of the 200 chemicals screened, 118 produced effects below baseline toxicity predictions, allowing the modeling of concentration-response curves and estimation of EC50s. 34 compounds exhibited toxic ratios (TR) > 10, i.e. provoking mortality below the predicted hydrophobicity-driven baseline toxicity Twenty-six compounds produced sublethal effects well below lethal concentrations or when no mortality was observed in the tested range of concentrations. From these compounds, ten high-TR chemicals with diverse modes of action and similar concentration-response slopes were selected for mixture testing. A mixture was designed to achieve equitoxic contributions of the individual test compounds at the predicted LC50 for the assumption of IA. Therefore, the ratios of individual compounds were based on the modelled LC10 values. Experimental mixture responses are compared with CA and IA predictions. A mass-balance model is applied to evaluate the relation of the mixture effects to the predicted baseline toxicity using predicted internal membrane concentrations. This study demonstrates that High-Content Zebrafish Embryo Screening, combined with specificity assessment, provides a strong foundation to advances prediction of mixture effects and supporting mixture risk assessment.

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1.09.B Fish Model Species in Human and Environmental Toxicology

1.09.B.T-01 Evaluating Steatosis Induction by Endocrine-Disrupting Chemicals in a Zebrafish Larval Model

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Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most common chronic liver disorder, affecting about 25% of the global population. The disease ranges from simple steatosis to metabolic dysfunction–associated steatohepatitis (MASH) and can progress to cirrhosis and hepatocellular carcinoma. Although MASLD is strongly linked to obesity and type 2 diabetes, many non-obese and lean individuals also develop the condition, suggesting that additional factors influence its onset and progression. Emerging evidence implicates endocrine-disrupting chemicals (EDCs) as potential contributors, though the underlying mechanisms remain poorly understood. To address this gap, we assessed the ability of ten EDCs to induce steatosis in a zebrafish larval model.

Transgenic zebrafish embryos expressing a liver-specific fluorescent reporter, Tg(fabp10a:EGFP), were exposed to non-developmentally toxic concentrations of EDCs from 5- to 9-days post-fertilization (dpf). Liver development and lipid accumulation were then evaluated using a neutral lipid dye and fluorescent microscopy. Preliminary results indicate effects on both liver-specific lipid content and liver development. However, our data indicate that none of the tested EDCs induced a steatotic phenotype at 9-dpf; p,p'-dichlorodiphenyldichloroethylene could only increase liver-specific lipids after 5-day exposures, whereas benzo[a]pyrene reduced liver-specific lipid content after both 5- and 9-day exposures. Furthermore, exposures to triphenyl phosphate and perfluorooctanoic acid increased fluorescence of the transgenic reporter linked to an intracellular lipid transporter at 9-dpf, suggesting disruption of lipid metabolism, while not showing any effect after only 5-day exposures. These findings highlight the importance of assessing these endpoints beyond the 5-dpf window. Ongoing studies are assessing the effects of 9-day EDC exposures combined with high-caloric diets, as well as liver-specific gene expression of MASLD-related genes at 5-dpf, to further elucidate the role of EDCs in MASLD initiation and progression.

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1.09.B.T-02 Multiple Endocrine Activities of Bisphenol Mixtures in Brain and Intestine of Zebrafish Embryos: Implications for Hazard Assessment

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Mixtures of endocrine-disrupting chemicals remain a major challenge for hazard and risk assessment, as current regulations underestimate their risks by assessing substances individually. In vitro mechanism-based assays are widely used to address this issue, yet in vivo tissue-specific endocrine responses to chemical mixtures remain poorly studied.

Herein, we aimed to determine whether bisphenol mixtures elicit tissue-specific endocrine effects in zebrafish embryos, focusing on receptor-regulated genes in the brain (cyp19a1b, regulated by zERs) and intestine (cyp3a65, regulated by zPXR, zAhR2 and zGR).

We first assessed the estrogenic and metabolic activity of 11 bisphenols using two transgenic zebrafish embryo assays: the EASZY assay (OECD TG 250), reporting brain expression of cyp19a1b, and the EMERGE assay, targeting intestinal expression of cyp3a65. All bisphenols except TCBPA induced cyp19a1b in the brain, and several also targeted cyp3a65 in the intestine, confirming their estrogenicity and revealing additional intestinal metabolic activity.

Based on single compound concentration-response data, we assessed a binary mixture of BPB and BPC using a ray design, and tested a six-bisphenol mixture at equipotent and environmentally relevant ratios. For the binary mixture, chemical analysis confirmed nominal concentrations and exposure stability. The BPB-BPC mixture produced additive effects on cyp19a1b and cyp3a65 expression in brain and intestine, respectively. For the six-bisphenol mixture, additive responses were observed at equipotent ratio in EASZY, while the mixture at environmentally relevant ratio led to two-fold lower effective concentrations than predicted by the concentration-addition (CA) model, suggesting a weak synergy.

By expressing median concentrations of the six bisphenols in European surface waters as BPA-equivalents, an interpretable and regulation-relevant metric, an estrogenic activity of 14.7 µg BPA-Eq/L was quantified, below the BPA freshwater environmental quality standard (129 µg/L) but above that for drinking water (2.5 µg/L). Together, these findings highlight the relevance of transgenic zebrafish embryos to capture endocrine activity of mixtures of compounds with similar modes of action in specific target cells and organs, while considering whole-organism toxicokinetics. This approach, combining biological assays with predictive models such as CA, supports improved mixture hazard assessment into a regulatory context.

Disclaimer/Disclosure:

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1.09.B.T-03 Assessing Molecular Responses to Groundwater-relevant Contaminant Mixtures in Zebrafish Eleutheroembryos: A Transcriptomic Approach for Effect-Directed Analysis

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As climate change exacerbates droughts, cities like Barcelona will increasingly rely on alternative water sources such as urban groundwater (UGW). However, UGW quality is compromised by wastewater and freshwater intrusion carrying pharmaceuticals, pesticides, and other micropollutants. This complex mixture may pose health risks poorly captured by single-chemical assessments, highlighting the need for effect-based monitoring and mechanism-focused tools. The zebrafish eleutheroembryo model provides a whole-organism perspective to detect sublethal and developmental effects, and, when combined with omics, reveals early molecular responses

and disrupted pathways. Transcriptomics in particular stands out for its sensitivity and genome-wide coverage, making it ideal for mixture toxicity assessment. Integrating transcriptomic biomarkers into effect-directed analysis (EDA) may enhance its sensitivity to help identify key toxicity drivers within complex mixtures. In this study, we exposed zebrafish embryos to a synthetic mixture of 77 organic compounds found in Barcelona's UGW, including pharmaceuticals, pesticides, herbicides, and industrial chemicals at concentrations ranging from 0.2 to 251 µg/L. Mortality and swim bladder inflation were recorded at 5 dpf, and transcriptomic analysis was performed on embryos from non-lethal doses. Target genes related to key toxicological pathways were analyzed using RT-qPCR. Mortality increased sharply at higher exposures, exceeding 80% above 33 µg/L. Swim bladder inflation emerged as a highly sensitive endpoint and was impaired at all concentrations, ranging from 30–63% inflation compared to controls (90±11%), with effects observed at environmentally relevant concentrations in Barcelona's UGW. While no significant changes were observed in genes related to thyroid disruption, neurodevelopment, and organ development, genes involved in xenobiotic metabolism responded markedly, including significant *cyp2k18* upregulation at 15 µg/L, preceding the onset of mortality. Non-monotonic responses were evident at intermediate doses in mortality, swim bladder inflation, and gene expression. RNA-Seq is currently being performed to identify additional sensitive biomarkers for EDA, and further work will expose eleutheroembryos to fractionated mixtures to validate the toxicants driving these effects. Ultimately, this approach aims to provide a sensitive, mechanistic tool for assessing complex mixtures in environmental monitoring.

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1.09.B.T-04 Zebrafish (*Danio rerio*) as a New Approach Methodologies (NAM)-model for Thyroid Hormone System (THS) Disruption-mediated Developmental neurotoxicity (DNT) – A case study with Methimazole

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Thyroid hormones (THs) are essential for brain development and neurodevelopmental processes, particularly myelin formation within nerve cells. Consequently, endpoints sensitive to developmental neurotoxicity (DNT) are critical when assessing the thyroid hormone signaling (THS)-disrupting potential of chemicals. Current DNT evaluations rely on rodent guideline studies (OECD TG 426 and TG 443), which are resource-intensive and require large numbers of animals, limiting chemical coverage and leaving no validated methods for environmental hazard assessment. In line with the 3Rs and the EU's roadmap to phase out animal testing, there is growing emphasis on developing New Approach Methodologies (NAMs). Zebrafish (*Danio rerio*) have emerged as a promising model in ecotoxicology and NAM development, as they are considered non-protected animals until free-feeding begins at 5 days post fertilization (dpf).

To address the need for sensitive NAM endpoints, we investigated methimazole (MMI) as a model THS-disrupting chemical and hypothesized that MMI would induce neurotoxic effects and brain developmental alterations through THS mechanisms. We present preliminary results on brain morphology changes following exposure to four sublethal concentrations of MMI. Quantitative analysis revealed significantly smaller brain size in 33-day-old zebrafish compared to controls (Student's t-test, $p = 0.0064$), with the optic tectum also significantly reduced ($p = 0.00002$). In contrast, no significant effects were observed at 5 dpf for either brain size or optic tectum. These findings suggest that zebrafish eleutheroembryos cannot predict brain alterations occurring at later life stages under the tested conditions. A full dataset will include additional brain regions such as the telencephalon, diencephalon, and myelencephalon.

We also confirmed THS disruption in both 5 dpf and 33 dpf zebrafish, evidenced by significant upregulation of the *tpo* gene. Transcriptional analyses of THS-related genes (*dio1*, *dio2*, *dio3a*, *thraa*, *thrb*) and DNT-relevant genes (*mbp*) will be presented, alongside immunohistochemical staining of thyroid follicular cells and myelin basic protein to evaluate the sensitivity of additional DNT markers.

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1.09.P Fish Model Species in Human and Environmental Toxicology

1.09.P-Mo036 Dietary Exposure to Bisphenol E Increased Abundance of Nocardia in Zebrafish Gut Microbiome

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Bisphenol A (BPA) is known for endocrine-disrupting effects and can alter immune responses and the gut microbiome, highlighting the need to evaluate its alternatives. Bisphenol E (BPE) is increasingly used as an alternative to BPA in consumer products, yet its biological effects remain largely unexplored. In this study, we investigate how long-term dietary BPE exposure affects intestinal microbial communities in zebrafish (*Danio rerio*).

Adult zebrafish (n=100) were exposed for 30 days to feed containing BPE, at 500 µg/kg (Low) or 5000 µg/kg (High), reflecting environmentally relevant and worst-case scenario. Intestinal content was collected, and the gut microbiome community structure was analyzed by 16S rRNA amplicon sequencing. Functional shifts in microbial metabolic production were also predicted.

Sequencing data revealed a statistically significant dose-dependent increase in the relative abundance of *Nocardia* species, including *N. asteroides*, *N. neocaledoniensis*, and *N. rhizosphaerihabitans* ($p < 0.001$, Kruskal-Wallis test). *Nocardia* are opportunistic pathogens causing nocardiosis, chronic granulomatous disease in fish, associated with gut dysbiosis, intestinal tissue damage and immune activation. Nocardiosis causes high mortality and has resulted in severe economic losses in aquaculture. Predicted functional changes in microbial metabolites were not statistically significant. However, genomic analyses of *Nocardia* spp. revealed high biosynthetic potential. *Nocardia*'s metabolites, including siderophores, may modulate surrounding microbiota and promote dominance of pathogenic *Nocardia*.

Although the overall gut microbiome community remained comparable across exposure groups, the robust increase in *Nocardia* reveals a biologically relevant shift that may have significant implications for gut health and host-microbiota interaction. This study provides the first evidence of microbiome alterations linked to BPE exposure, suggesting potential implications for fish health and immune regulation. The findings highlight the need for further evaluation of BPA substitutes which may contribute to risk assessment.

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1.09.P-Mo037 Perfluorooctanesulfonamide-induced epiboly delay is associated with decreased ATP production within zebrafish embryos

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Per- and polyfluoroalkyl substances (PFAS) have been widely used within various industrial processes and consumer products, such as food packaging and fire-fighting foams. PFAS persistence within environmental media has led to ubiquitous exposure in humans and wildlife. We previously conducted a high-content toxicity screen of 38 different PFAS within the first 72 h of zebrafish development. This screen identified perfluorooctanesulfonamide (PFOSA) as the most potent developmental toxicant among the 38 PFAS tested. At non-lethal concentrations, exposure to PFOSA from 0.75 to 6 h post-fertilization (hpf) – a developmental window that includes cleavage, blastulation, and early-gastrulation – resulted in a concentration-dependent delay in epiboly (epiblast cell migration) by 6 hpf. Therefore, for this study, we determined whether 1) PFOSA delays epiboly similar to Cytochalasin B (CCB), a potent actin polymerization inhibitor, by inhibiting actin polymerization within the yolk, as epiboly progression is reliant on yolk-associated actin networks, and 2) PFOSA-induced effects are associated with a decrease in embryonic ATP, as PFOSA is a potent uncoupler of oxidative phosphorylation in vitro. To accomplish these objectives, we employed zebrafish embryo-based chemical exposures, liquid chromatography-mass spectrometry, phalloidin-based staining of F-actin, laser-scanning confocal microscopy, mRNA-sequencing, and luminescence-based ATP assays. Although PFOSA and CCB induced a similar magnitude of epiboly delay beginning at 4 hpf following initiation of exposure at 0.75 hpf, PFOSA did not, contrary to CCB, significantly decrease yolk-associated actin within embryos. Using

mRNA-sequencing, we found that, consistent with chemical-specific differences in actin polymerization effects, PFOSA-exposed embryos were also transcriptionally different from CCB-exposed embryos. Moreover, phenotypically matched, PFOSA-exposed embryos at 6 hpf were transcriptionally similar to vehicle-exposed embryos at 5 hpf, and PFOSA delayed the maternal-to-zygotic transition (MZT) beginning at 5 hpf. Finally, PFOSA-induced epiboly delay was associated with decreased ATP levels in vivo, an effect that was partially mitigated by co-exposure to exogenous ATP. Overall, our findings suggest that PFOSA exposure during early embryonic development decreases ATP production in vivo without affecting actin polymerization, an effect that was associated with PFOSA-induced delays in epiboly and the MZT.

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1.09.P-Mo038 New approach methodologies for aquatic toxicity testing – Omics enhanced Fish Embryo Test (E-FET)

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The REACH regulation requires chronic fish toxicity testing, such as the Fish Early Life Stage (FELS) test, which is resource-intensive and reliant on a high number of vertebrate animals. To support the development and regulatory uptake of animal-free new approach methods (NAMs), this study examined whether transcriptomic points-of-departure (tPODs) derived from zebrafish embryos can serve as protective and predictive indicators of chronic toxicity for 16 industrial chemicals with differing modes of action and sensitivity profiles. Using an enhanced Fish Embryo Toxicity test (E-FET) for each substance zebrafish embryos were exposed to five sublethal, analytically confirmed concentrations spanning the NOEC–LOEC range reported from FELS studies. Subsequent transcriptomic profiling, including RNA sequencing, differential gene expression analysis, and benchmark dose (BMD) modeling via DRomics, enabled the derivation of gene-level concentration–response relationships and tPOD values. The majority of chemicals that induced growth or survival effects in FELS also produced clear, concentration-dependent transcriptional alterations in the embryos, and the resulting tPODs generally mirrored FELS NOECs, with particularly strong alignment for substances primarily affecting growth. Chemicals that produced no observable effects in FELS similarly showed no transcriptomic signal in the E-FET, supporting the relevance and sensitivity of the tPOD approach. For two substances, tPOD derivation was not possible due to rapid degradation in the test medium resulting in insufficient exposure, underscoring the necessity of rigorous test concentration validation and application testing - particularly for substances with low solubility, high adsorption, or instability. These findings further indicate that semi-static exposure designs may be inadequate for certain chemical classes and that alternative systems should be considered to ensure stable exposure conditions. Collectively, the study provides strong evidence that zebrafish embryo tPODs can serve as early, protective indicators of chronic fish toxicity, while also highlighting current methodological limitations and the need for broader chemical evaluation to fully substantiate their utility for regulatory decision-making.

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1.09.P-Mo039 Identification of Biomarkers for Immunosuppression in PAMP-challenged Zebrafish Embryos using Transcriptome Analyses

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It is assumed that in stressed ecosystems, pathogens exert strong pressure on populations. In addition, the organisms in the environment are exposed to a high number of environmental pollutants. The ecotoxicological hazard and risk assessment of chemicals do 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. 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 project aims at the identification of reliable markers for the MoA of immunomodulatory chemicals in zebrafish (*Danio rerio*). For this, the classic zebrafish embryo toxicity test was adapted and the chemical exposure is followed by an immune challenge, which enables the investigation of chemical effects in the activated immune system. In a first step, a suitable Pathogen-associated-molecular-pattern (PAMP) that induces an immune system activation, was identified. In four independent experiments the PAMP flagellin was shown to induce a specific and reproducible immune response.

In the main experiments, freshly fertilized zebrafish eggs are exposed to a chemical for 48 h followed by an immune challenge via flagellin injection. After 3 h, the RNA of the exposed embryos is extracted and a transcriptome analysis is performed. In the first experiment with the immunosuppressive drug clobetasol propionate (CP) as exposure chemical, the flagellin treatment as well as the CP-treatment affected mostly genes involved in immune processes. The differentially expressed genes (DEGs) of the PAMP exposure were compared to the DEGs of CP-PAMP and sorted into hyper (stronger differential expression in CP-PAMP than in PAMP treatment), hypo (lower differential expression) and non-responsive genes (consistent expression). Interestingly, the hypo-responsive genes which are mostly involved in immune processes reveal an additive effect of CP and PAMP, indicating that CP suppressed the immune system activation by flagellin.

The data of all five experiments will serve to identify reliable markers for immunosuppressing MoA after immune system activation, which could be integrated into classic ecotoxicity testing.

1.09.P-Mo040 Integrating Molecular and Behavioral Endpoints to Assess neurotoxicity of BPA Alternative Compounds

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Understanding the link between molecular endpoints and phenotypic outcomes is crucial in ecotoxicology to establish adverse outcome pathways and better predict environmental risk. This study aims to identify correlations between molecular-level responses and behavioral impairments following contaminant exposure. Thus, transcriptomic changes and alterations in locomotory behavior in zebrafish (*Danio rerio*) early life stages were assessed after exposure to BPA and BPA alternatives BPAF and BPZ. Furthermore, the sensitivity of two exposure protocols was compared: a continuous 120-hour exposure starting at ~ 3 hours post-fertilization (hpf), and a shorter 24-hour exposure at 96 hpf larvae. The shorter exposure targets a stage when the chorion is no longer present, potentially reducing confounding effects that may occur during early embryonic exposure. We hypothesize that the 24-hour larval exposure may demonstrate equal or greater sensitivity due to direct contaminant interaction without the protective chorion barrier. Results from this comparative approach will inform best practices for zebrafish toxicology testing. This work will further enhance our understanding of BPAF and BPZ mechanisms of action and consequent threats for ecosystem health.

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1.09.P-Mo041 Toxic Legacies: Ecotoxicity of Phenylarsenical Chemical Warfare Agents and Their Transformation Products in Freshwater and Brackish Model Organisms

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Approximately 50,000 tons of chemical warfare agents (CWAs) were dumped in the Baltic Sea after the World Wars, which was considered the most efficient and safest disposal method at the time. Progressive corrosion of metal shells and casings results in the release of CWAs into the environment. Originally designed to cause physiological harm to humans, CWAs also pose a significant threat to aquatic ecosystems. Current environmental risk assessment of CWAs is primarily based on in silico analyses and a limited number of in vivo studies, with a predominant focus on intact CWAs. However, emerging evidence highlights that transformation products can also be considerably toxic to exposed biota. Given the rather scarce knowledge on CWAs and their transformation products toxicity, it is essential to develop a customized, multi-level bioassay battery for a holistic ecotoxicological assessment. Arsenic-containing agents are among the most commonly dumped CWAs. This project aims to evaluate the ecotoxicological effects of the CWA-related phenylarsenicals Clark 1 and phenyl(dichloro)arsine and their methylated transformation products methyldiphenylarsine oxide, methylphenylarsinic acid and dimethylphenylarsine oxide. All CWAs selected have previously been detected in Baltic Sea sediments. Freshwater and marine model organisms spanning different trophic levels are used (*Danio rerio*, *Gammarus fossarum*, *Oryzias melastigma*, *Gammarus oceanicus*). By extending beyond traditional freshwater test species, this approach aims for a more ecologically representative toxicity profile for marine and brackish ecosystems. To evaluate early developmental alterations and to establish acute toxicity thresholds, the fish embryo toxicity (FET) test (with *D. rerio* & *O. melastigma*) was conducted in accordance with the OECD No. 236 guideline. FET-derived thresholds are furthermore used to define non-lethal, yet biologically and environmentally relevant effect concentrations for neurotoxicity testing, addressing a relevant mode of action. Systemic identification of neurotoxic pathways is achieved by linking key events at the biochemical, tissue and organismal level with state-of-the-art techniques. Expected outcomes include novel insights into neurobehavioral effects, particularly locomotor impairment in early life stages of *D. rerio*. Ultimately, this research seeks to enhance ecosystem-based risk assessments and drive more effective environmental management strategies for sea-dumped CWAs.

1.09.P-Mo042 Chronic Fish Toxicity Case Studies toward a Best Practice Guidance Document

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Chronic fish toxicity data form part of aquatic safety assessments and are mandated under frameworks such as EU REACH. However, traditional in vivo methods rely heavily on animal testing, are resource-intensive and provide limited information on a few model organisms to protect the environment holistically. In line with the European Commission's roadmap to phase out animal testing and priorities highlighted by ECHA in their KARC 2025 document, there is growing momentum to implement New Approach Methodologies (NAMs) within Next Generation Risk Assessment (NGRA) for chronic aquatic toxicity.

This International Collaboration on Cosmetics Safety (ICCS)-led project aims to address standard information requirements for chronic fish toxicity by evaluating the applicability of NAMs through multiple case studies focused on cosmetic ingredients. Insights from these case studies will be consolidated into an ICCS best practice guidance document to help regulatory and industry safety assessors.

The work is driven by a tiered approach: starting with identification of data gaps and developing solutions to support safety assessments and regulatory registration dossiers (e.g., under REACH), ultimately progressing towards applying environmental Next Generation Risk Assessment (NGRA).

The ICCS project is closely aligned with case studies led by other organizations, ensuring cross-fertilisation of approaches and harmonisation of data integration strategies. Together, these efforts aim to inform and shape future OECD Integrated Approaches to Testing and Assessment (IATAs) for chronic aquatic toxicity, promoting global regulatory acceptance of NAM-based frameworks.

The poster will provide an initial project overview and outline planned case studies to foster discussion and collaboration with the SETAC community. Ultimately, this work supports regulatory acceptance of NAMs and NGRA, advancing environmental protection anchored on modern science.

1.09.P-Mo043 Advancing Safety Assessment of Redox Flow Battery Electrolytes Using Zebrafish and Aquatic Models: Multi-Endpoint Toxicological Insights

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Following the initial findings presented previously, this study expands the toxicological and ecotoxicological evaluation of quinone-based redox-active compounds used in flow battery systems. A multi-endpoint strategy was employed using new alternative models—Danio rerio, Daphnia magna, and Raphidocelis subcapitata—to assess developmental toxicity, endocrine disruption, and environmental impact. In the case of zebrafish, the protocols were extensively validated at Biobide. The ecotoxicological studies conducted are based on internationally recognized guidelines, such as the OECD Test Guidelines 201 and 202 for acute tests in algae and daphnia.

Four structurally distinct aminoquinones were tested. All compounds showed signs of teratogenicity in zebrafish embryos, with MHQ-DMA and MHQ-Morph classified as likely teratogenic based on teratogenic index values. In contrast, AQDS-Na was considered non-toxic under the tested conditions. Thyroid disruption assays revealed no goitrogenic effects for most compounds, except for MHQ-DMA, which induced a statistically significant reduction in thyroid fluorescence. Bioavailability analysis showed low internal absorption (<10%) in zebrafish larvae, suggesting that observed effects may be driven by surface interactions or indirect mechanisms rather than systemic exposure.

Ecotoxicological assays demonstrated that AQS-Na, MHQ-DMA, and MHQ-Morph were toxic to Daphnia magna, causing significant immobilization at low concentrations. All four compounds inhibited algal growth in Raphidocelis subcapitata, with EC50 values in a comparable range, indicating consistent toxicity across species. These results highlight the importance of integrating multiple biological endpoints to comprehensively assess the emerging battery materials' safety profile. The findings support the application of Safe- and Sustainability-by-Design (SSbD) principles in the development of next-generation energy storage technologies.

KW: teratogenicity, thyroid disruption assay, ecotoxicology, Zebrafish, Daphnia magna, Raphidocelis subcapitata, NAM.

Disclaimer/Disclosure:

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1.09.P-Mo044 Analyzing the Metabolic Effects of Nitrobenzene Exposure in Japanese Medaka (Oryzias latipes) with Consideration of Spawning 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. However, based on the current standards, the damage can be recognized only visually identifiable damaged fish. 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 spawning 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 spawning cycle and nitrobenzene exposure on the extracorporeal metabolites of medaka was undertaken. distinctive identification of metabolites that exhibited a notable decrease after nitrobenzene exposure, as compared to their pre-exposure state, indicated an encompassing influence on the spawning cycle. Using the Mummichog pathway analysis algorithm, we identified significant pathways in our dataset. As a result, pathways found to be affected by nitrobenzene were, in order, the steroid synthesis pathway, xenobiotics metabolism, and lipid synthesis. Subsequently, we identified biomarkers to differentiate changes attributed to the spawning cycle and those induced by nitrobenzene exposure.

Disclaimer/Disclosure:

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1.09.P-Mo045 Automatization of the EASZY assay (OECD TG N° 250): A Robust Imaging Platform for the screening of Environmental Endocrine Active Substances

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The TG OECD N°250 (EASZY assay), adopted in 2021, described a mechanism-based screening method designed to detect environmental endocrine active substances acting as agonists through estrogen receptors (ERs) to induce the expression of the ER-regulated cyp19a1b expression in transgenic tg(cyp19a1b:GFP) zebrafish embryos(1).

In this transgenic model, the GFP expression faithfully reports the endogenous cyp19a1b expression within the brain allowing to quantify in a noninvasive way the expression of this gene using fluorescence imaging.

The standard EASZY assay protocol involves exposing fertilized embryos (up to 4 hours post-fertilization (hpf)), to test substances for a period of 96 hours. Following this exposure, the fluorescence in the brain of the exposed organisms is measured using a fluorescence microscope (2). This imaging step presents a practical challenge. It required the manual positioning of each 96 hpf fry to obtain a clear dorsal image of the brain. This manual process is time-consuming and could introduce variability into the results inconsistent fry orientation. To improve this step, we applied an automated imaging strategy. This system integrates a VAST Bioimager (Union Biometrica Inc.) combined with a fluorescent microscope. The VAST Bioimager greatly facilitates precise and reproducible positioning of individual zebrafish fry through its rotative capillary mechanism. Additionally, we adapted the EASZY ImageJ macro to enable the analysis of Z-stack images, ensuring objective data extraction from the acquired fluorescent images.

Validation of this automated methodology was conducted using the 11 compounds from the OECD inter laboratory validation Assay (4). Our results indicate that the automated assay demonstrates sensitivity comparable to that of the established manual intercalibration study. A significant advantage of this automation is the improved reproducibility in fry positioning. This led to a measurable decrease in the standard deviation associated with the quantification of fluorescent intensity across batches of 24 fry exposed to identical experimental conditions.

In summary, the automated imaging method described here offers several key benefits for the OECD250 EASZY assay. It provides time savings in the imaging workflow, ensures a more reproducible positioning of the fry, while reducing the variability of GFP quantification which increase the statistical robustness of the data.

1.09.P-Mo046 Long-term toxicity to fish : building a weight of evidence approach

Yannick Bayona, Giacomo Grassi and Véronique Poulsen, L'Oréal, France

Different chemical regulations worldwide require that information on fish ecotoxicity is provided. This includes long term effects on fishes, depending on specific requisites, which have been historically derived from in vivo chronic toxicity assays, using juveniles or adult fishes. In line with the 3R objectives and the European Directive on the protection of animals used for scientific purposes, this use-case presents a weight of evidence (WoE) approach to assess the long-term ecotoxicity to fish, as an alternative to in vivo fish chronic studies. We propose an approach combining available information and generating additional data to build a robust WoE to cover long term toxicity to fish. To do so, we integrate a range of acute ecotoxicity endpoints, both already available and generated ex-novo, including the acute fish embryo toxicity assay (OECD TG 236), the RT-Gill assay (OECD TG 249) and historical fish acute toxicity data (OECD TG 203). In parallel, we leveraged information from the Endocrine Disruption test EASZY (OECD TG 250), in which additional observations were collected, such as morphological and physiological traits, as an indication of potential sublethal effects. The approach also includes interspecific effects comparison to complement fish data, such as ecotoxicity studies covering short- and long-term effects on freshwater invertebrates and algae. A Quantitative Structure Activity Relationship (QSAR) is also used to predict the chronic fish ecotoxicity as well as the identification of specific mechanisms of action based on chemical structure. Finally, the molecular properties that may drive specific physico-chemical behaviour (hydrophobicity, ionicity) are included in the approach. The rationale is built upon the analysis of acute-to-chronic ratios (ACRs), interspecies correlations, physicochemical properties, and sublethal effects, as a mean to cover long term toxicity to fish. We propose to develop a framework based on a range of lines of evidence to support whether chronic fish effects are expected or not, also providing an overview of the strengths and limits of our approach. Ultimately, by proposing a synthetic approach, we strive to contribute to

paving the way towards a comprehensive framework to determine when chronic fish testing could be considered necessary or conversely avoided for regulatory assessment.

1.09.P-Mo047 Developmental Toxicity and Transcriptional Changes in Zebrafish Embryos following Exposure to PM2.5 Extracts

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Particulate matter $2.5 \mu\text{m}$ (PM2.5) is a major environmental concern in Korea, despite national regulations and seasonal management policies. Its health effects, particularly during winter, remain a significant issue. In this study, we examined the developmental toxicity of PM2.5 using a dechorionated zebrafish embryo model, focusing on phenotypic changes and transcriptional alterations in thyroid and neuronal genes. PM2.5 samples were collected from December 2024 to February 2025 in Korea, representing high-concentration winter exposure scenarios. PM extracts were prepared using an organic solvent mixture, diluted in E3 medium containing 0.01% dimethyl sulfoxide, and applied to embryos from 4 hours post-fertilization (hpf) to 5 days post-fertilization (dpf). Morphology and gene expression were assessed. PM2.5 exposure caused developmental abnormalities in a concentration-dependent manner. Embryos at the highest exposure level (300 $\mu\text{g/mL}$) exhibited marked pericardial edema, reduced body length, shortened jaw structure, and smaller eye size. The thyroid-related gene *dio2* and the neural development gene *mbp* were downregulated, indicating disruption of endocrine balance and neurodevelopmental processes. These findings suggest that Korean PM2.5 extracts can induce developmental toxicity and impair thyroid-mediated neurogenesis. Chemical analysis identified abundant ionic species, including NO_3^- , SO_4^{2-} , and NH_4^+ . Further studies are needed to identify specific toxic constituents in the PM extracts and to understand the consequences of transcriptional changes in key genes in zebrafish.

Disclaimer/Disclosure:

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1.09.P-Mo048 Modelling Chronic Toxicity to Fish Considering Data Uncertainty

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Chronic toxicity to fish is a crucial regulatory endpoint; however, experimental NOEC studies are costly and time-consuming and raise ethical concerns. This study develops two complementary *in silico* methods for predicting chronic fish toxicity while explicitly accounting for data uncertainty. Approach 1 creates acute-to-chronic QSAR models based on an enhanced Verhaar mode-of-action (MoA) and adds alerts for protein and estrogen receptor reactivity. Linear regressions were derived for MoA1–MoA3; however, due to limited data and regulatory considerations, MoA4 and MoA5 were excluded. MoA1 and MoA2 were later integrated into a model supported by mechanistic, structural, and parametric applicability domains. Approach 2 uses a two-step read-across/trend-analysis workflow that first assigns compounds to the augmented Verhaar MoA and then finds structural analogs for prediction. Variability in experimental NOEC values was modeled using a Gaussian distribution ($\sigma = 0.67$), while QSAR prediction uncertainty was represented with Student-t distributions. Together, these methods provide chemical-specific uncertainty ranges for NOEC estimates. Internal 1000-fold cross-validation demonstrated strong model performance. Correlations between acute and chronic toxicity were high for MoA1 and MoA3 ($R^2 = 0.84$) and acceptable for the combined MoA1+MoA2 model ($R^2 = 0.74$). Predicted NOEC distributions were transformed into probabilistic GHS chronic hazard categories, enabling grey-zone classification when uncertainty prevents definitive categorization. All workflows were implemented in the QSAR Toolbox. By integrating experimental and model uncertainties, this approach enables probabilistic GHS classification rather than fixed categories, reflecting real data variability and reducing misclassification risk. This better reflects the variability of real data and lowers the risk of misclassification. These workflows also support 3R principles (Replacement, Reduction, and Refinement) by offering transparent, reproducible, and robust alternatives to chronic fish testing, suitable for preliminary hazard screening and regulatory prioritization.

1.09.P-Mo049 Challenges in Applying OECD 210 Validity Criteria: Refinement Opportunities

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The OECD Test Guideline (TG) 210 for the Fish Early-Life Stage (FELS) test is the standard method recommended under Annex X of the European Union REACH Regulation for fulfilling the long-term fish toxicity requirement. It specifies strict validity criteria and minimum fish numbers to ensure robust data. However, practical implementation often reveals challenges in fulfilling these criteria without compromising animal welfare or data integrity.

This presentation explores critical gaps in the application and interpretation of OECD 210 validity items, focusing on embryo quality, vague or inconsistent definitions of malformation, and their implications for study validity. The current guidance lacks clarity regarding acceptable levels of malformation in embryos and larvae and their relationship with final survival outcomes, as referenced in TG 210 paragraph 29 on abnormal appearance. These uncertainties frequently result in inconsistent validity assessments across laboratories and, in some cases, unnecessary repetition of animal testing. Additionally, such inconsistencies could lead to ecotoxicity data that misrepresent actual hazard potential, resulting in unreliable risk assessments and regulatory decisions.

Through analysis of fish embryo studies in fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*), we identify examples of sublethal findings and humane endpoints that illustrate the limitations of current validity criteria. Specifically, we highlight how clearer definitions of malformation thresholds (expressed as acceptable percentages), refined humane endpoints for euthanasia, and improved consideration of sublethal effects with potential or non-potential for regeneration could enhance both data quality and animal welfare. We propose that revision of the OECD 210 guideline should include explicit, evidence-based validity parameters that integrate malformation rates with survival and abnormal appearance criteria, while incorporating humane endpoints to minimise animal suffering. These refinements would improve the consistency of test interpretation across laboratories, reduce the need for repeated testing, and better align regulatory practice with the 3Rs principles of replacement, reduction, and refinement.

By identifying these opportunities, this work aims to support the scientific and regulatory dialogue on updating OECD 210 validity criteria to ensure robust, ethical, and reproducible fish early-life testing under regulatory requirements.

Disclaimer/Disclosure:

This presentation is intended for a professional and regulatory audience only

- The content shared is based solely on publicly available information, including Shell's published Animal Welfare Report.
- No confidential, proprietary, data is included.
- This session is not open to media or press representatives.
- The views expressed are for informational purposes and do not constitute official policy statements unless explicitly stated.

1.09.P-Mo050 The SEASON Project: How Will Climate Change Affect the Risk Associated With Sediments Contaminated With Organic and Inorganic Pollutants?

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Sediments are both a sink and a source of various organic and inorganic pollutants. In the SEASON project we aim to investigate how environmental changes (temperature, pH, and remobilization) affect the fate, availability, and toxicity of these pollutants. Additionally, by investigating how these changes influence sediment processes and microbial communities, we will develop models to predict the impact of these pollutants on aquatic ecosystems under climate change conditions.

The SEASON project consists of four different subprojects, three of which focus on studying the effects of metals, PFAS, and pollutant mixtures. These subprojects consist of various in vivo and in vitro assays, as well as aquatic microcosm simulations that can regulate climate change related parameters. The fate, bioavailability, and toxicity of pollutants in sediment will be studied, including microbiome and chemical analysis. The aim of the fourth subproject is to refine and develop predictive models for improved risk assessment under climate change conditions.

Zebrafish embryos have been used to investigate teratogenicity, developmental toxicity, behavioral changes, and

gene expression alterations caused by contaminated sediments using a contact assay. Based on these results, two sediments will be chosen for conducting the microcosm studies, in which various toxicological effects in adult zebrafish, including reproduction and behavior, will be assessed. Changes in temperature and pH will mimic climate change conditions, while remobilization events will be introduced to simulate heavy rain events. Microbiome analysis will be performed using different sequencing technologies not only on sediment samples, but also on fecal and intestinal samples from the fish. Chemical analyses will quantify PFAS, metals, and their speciation throughout the study. Effect-directed analysis (EDA) will be used to identify key toxicants in sediments used for microcosm studies. The subsequent creation of a predictive model will increase the understanding of the impact of climate change on sediment contamination and aquatic ecosystem health.

Disclaimer/Disclosure:

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1.09.P-Mo051 Characterization of Thyroid Transmembrane Transporters in the Brain: Advancing the Utility of Zebrafish Embryos for Thyroid Hormone System Disruption Studies

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Transmembrane transporters at the blood-brain and brain-cerebrospinal fluid barriers are vital regulatory components of the intricate thyroid hormone (TH) system allowing for adequate supply of THs to the developing brain. Disruption of these transport systems can impair neurodevelopment in the embryonic brain and lead to adverse, irreversible outcomes in affected organisms. Improved mechanistic insights and better testing systems for endocrine disrupting chemicals (EDCs) targeting TH transport are therefore critical to protect human and environmental health.

A wide range of mammalian TH transmembrane transporters (THTMTs) belonging to the group of solute carrier proteins have previously been characterised in rodent models, overexpression cell lines and recently in a novel choroid plexus organoid model. These THTMTs belong to the families of monocarboxylate transporters (e.g. MCT8, MCT10), organic anion transporters (e.g. OATP1C1), and cationic amino acid transporter (e.g. LAT1, LAT2).

To study chemical effects on THTMTs, interspecies differences and in vitro to in vivo extrapolations are an ongoing challenge. While human cell-based models are preferred for studying human THTMT disruption in vitro, these models lack the in vivo complexity of a whole-organism vertebrate model.

Zebrafish (*Danio rerio*) embryos seem to be a promising model for studying THTMT dysregulation, with the potential to bridge the gap between in vitro work and studies of higher vertebrate organisms. In contrast to rats, MCT8 deficient zebrafish larvae display a phenotype similar to that of human patients with MCT8 mutations, suggesting conservation of important thyroid transport functions. Still, the expression patterns and spatial distribution of THTMTs in zebrafish remain largely unexplored.

In this recently started PhD project, the initial focus is on characterizing zebrafish orthologs for known human THTMTs. Gene expression pattern in zebrafish embryos will be assessed by qPCR, and their spatial localisation in the embryonic brain will be mapped via in situ hybridisation. This foundational characterisation will support future investigations of the impact of potential TH disruptors in different transgenic zebrafish lines.

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1.09.P-Mo052 Rethinking Chronic Fish Toxicity Testing: Case Studies Toward an Integrated Approach to Testing and Assessment

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Assessment of aquatic toxicity is a cornerstone of environmental hazard and risk assessment under various regulatory frameworks. Chronic fish toxicity data are often required, with fish serving as a representative predator species for the pelagic environment. The Fish Early Life Stage (FELS) test (OECD Test Guideline 210) is the standard method for generating such data. However, the FELS test involves the use of hundreds of vertebrate animals, raising ethical and regulatory concerns, and typically relies on a limited number of standard species, creating uncertainty regarding the protection of more sensitive or threatened species.

In line with the EU REACH Regulation's requirement to make full use of existing information before conducting new vertebrate tests, there is growing interest in approaches that combine existing data and non-animal methods. These include the integrated use of read-across, acute fish toxicity data, non-vertebrate test methods (e.g., Daphnia assays), and New Approach Methodologies (NAMs) such as *in silico* predictions and *in vitro* fish cell line assays. Such approaches can strengthen predictive power in hazard and risk assessments while supporting the goal of reducing vertebrate testing.

Although tiered testing frameworks and integrated testing strategies (ITS) for fish toxicity exist, practical examples and a dedicated Integrated Approach to Testing and Assessment (IATA) for chronic fish toxicity are still lacking. Concurrently, new *in vitro* methods for assessing chronic fish toxicity are under development.

A group of experts coordinated by ECETOC are developing a series of case studies to explore how existing data, including non-vertebrate methods and NAMs, can be integrated to conclude on chronic fish toxicity. The case studies aim to cover a range of chemical types, uses, and industry sectors, as well as different regulatory contexts, such as risk assessment and classification and labelling. The intention is to assess data-rich and data-poor substances, and some case studies may include existing FELS data. This presentation will provide information on the case study substances, their regulatory and scientific context, and initial findings from the ongoing work.

The outcomes from this work are expected to inform regulatory and industry decision-making, potentially contribute to the development of OECD guidance, and support the broader European Commission Roadmap toward phasing out animal testing in chemical safety assessment.

1.09.P-Mo053 Distinct ecological risks associated to geogenic and smelting metal contamination.

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Metals and metalloids in aquatic ecosystems pose ecological threats to resident biota. This study evaluated 27 metal/metalloids in the water, sediment, and gills of *Oreochromis niloticus* collected in two coastal lagoons (Maembá and Carapebus) impacted by metallurgical activities and an aquaculture facility (Alegre) influenced only by natural geogenic inputs. The following were analyzed in the gills: the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-S-transferase (GST); the non-enzymatic defense molecules glutathione (GSH) and metallothionein (MT); oxidative stress biomarkers (lipid peroxidation, protein carbonylation, and DNA strand breaks); histopathological lesions; and the density of mucous cells (MC). The concentrations of the metals/metalloids varied in the water and sediment of all sites. The gills of fish from the Maembá and Carapebus exhibited high concentrations of Al, V, Cr, Zn, and Sr; high SOD, CAT, GPx and GR activities; GSH depletion; pronounced oxidative damage (protein carbonylation and DNA strand breaks); extended histopathological lesions; and a high density of MC. The gills of fish from Alegre showed high levels of Fe, Ba, Rb, and Ce; elevated MT; and only focal histopathology; they could clearly be differentiated from the samples from impacted lagoons. This study provides novel evidence that metallurgical activities (smelting) can chemically modify metals and thus enhance their biological impact compared with natural geogenic sources. The combined use of biochemical and morphological biomarkers allowed for effective discrimination of different effects revealing distinct ecological risks associated with metal/metalloid contamination of different origins in aquatic ecosystems

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1.09.P-Mo054 Integrating New Approach Methodologies to Inform Chronic Fish Hazard: A HESI Case-Study

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Regulatory demands for chronic fish early-life stage (FELS; OECD TG 210) data are increasing across sectors, yet default FELS testing can be resource-intensive and require substantial numbers of animals. Multiple new approach methodologies (NAMs) relevant to chronic aquatic toxicity have been developed and, in some cases, standardized (e.g., fish embryo and gill cell assays, hepatic biotransformation), but practical guidance for integrating these data into fit-for-purpose decisions remains limited. The HESI Alternatives to Chronic Fish Testing Working Group is developing a flexible, weight-of-evidence framework to evaluate when confidence in NAM-based evidence is sufficient to meet chronic fish data needs, and when in vivo chronic fish testing remains necessary. The framework is organized in two phases: (1) prioritizing whether chronic fish toxicity testing is needed given external and internal exposure potential and existing evidence; and (2) if chronic data are required, integrating diverse lines of evidence to estimate chronic endpoints and associated uncertainty. Potential key lines of evidence that could be incorporated into the framework include physicochemical and environmental fate data, in silico predictions (e.g., QSARs), acute-to-chronic ratios, invertebrate chronic data, existing in vitro assays (e.g., RTgill, fish embryo toxicity test), available mechanistic high-throughput data (e.g., ToxCast), and emerging endpoints such as transcriptomic points of departure.

To demonstrate feasibility and identify framework roadblocks, we are applying the approach to case studies. The chemicals of interest for case studies are selected based on the availability of robust in vivo chronic data and NAM data, enabling direct comparison of NAM-informed conclusions with in vivo ELS outcomes.

Ongoing analyses will evaluate how lines of evidence can be integrated together and how uncertainty shifts with data availability and agreement. Expected outputs include a transparent decision workflow and documented case studies to support consistent, chronic fish hazard and risk assessments.

1.09.P-Mo055 Assessment of (Neuro)developmental Effect Signatures of Organophosphate and Neonicotinoid Pesticides Using Zebrafish High-Content Morphology and Behavior Analysis

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Legacy organophosphates and modern neonicotinoids are still used globally despite region-specific restrictions. For many of these compounds, a knowledge gap exists regarding their potential developmental or neurodevelopmental effects, particularly during early life stages. This is further challenged by the frequent co-occurrence of pesticide mixtures in the environment, with scarce monitoring and mechanistic toxicity information available. To address this gap, we employed New Approach Methodologies (NAMs) using the zebrafish embryo as an alternative model. The aim of the study is to generate comprehensive, developmental toxicity and neurotoxicity fingerprints that can be compared to an existing reference library to predict underlying mode of action information. Zebrafish embryos were exposed to chlorpyrifos-oxon (CPO) from 0 to 4 days post-fertilization (dpf). At 4 dpf, automated imaging was performed, and a suite of morphological endpoints were quantified using FishInspector. Exposure to 0.03-5 μ M of CPO resulted in a range of concentration-dependent developmental toxicity effects. Quantitative analysis of morphological endpoints revealed a specific signature, characterized by pronounced reductions in swim bladder area ($AC_{50} = 0.15 \mu$ M), eye area ($AC_{50} = 4.93 \mu$ M), and a concomitant increase in yolk sac area ($AC_{50} = 0.95 \mu$ M). In contrast, mortality remained low (~25%), even at 5 μ M and there were no significant changes in heart rate, arrhythmia, or blood circulation. Morphology assessments and neurobehavioural profiles, captured with the VAMR assay, which assesses locomotor activity, startle responses, habituation learning, and memory retention at 5 dpf, will be performed for a range of organophosphate and neonicotinoid compounds. The next phase of this study will focus on prioritized organophosphates and neonicotinoids to compare potential class-specific morphological and behavioral fingerprints.

1.09.P-Mo056 Development of a Zebrafish Metabolome Atlas from Different Transgenic Lines with a Focus on Steroid Hormones and Lipids to Unravel the Effects of Endocrine Disruptors

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Zebrafish (*Danio rerio*) embryos are a well-established and important model in the research of endocrine disrupting chemicals (EDCs). They have many advantages like the homology to the human genome and a very high number of genes that can also be associated to human diseases. Furthermore, the simplicity of creating transgenic lines allows for even more focused and accessible research on specific biomarkers in (eco)toxicological and metabolomic studies including endocrinology. Moreover, these transgenic zebrafish are increasingly used for the assessment of EDCs in research and in a regulatory context. For example, different established and currently validated OECD test guidelines use transgenic fish lines to assess EDC-specific effects in embryonic life-stages. In the context of new approach methodologies (NAMs) and the EU roadmap for phasing out animal experiments, these models gain increasing importance. However, basic differences between zebrafish lines remain insufficiently described. To investigate if there are detectable and significant variances in the early life stage metabolome between different transgenic lines we compared various metabolite levels (lipids, steroids, neurotransmitters) of the wildtype line, a triple-transgenic line tg(eno2:GFP), gfap:mCherry, mbp:mCitrine (nervous and glial cells) and the tg(tg:mCherry) (thyroid follicles) line at multiple timepoints up to 5 days post fertilization. Samples of embryos were extracted and analysed using liquid chromatography combined with high resolution mass spectrometry. Based on the results we will focus on one of these lines to further explore how metabolites, incl. hormone levels differ throughout early life development. Embryos will also be exposed to EDCs to study how metabolite levels are influenced at different concentrations, and to increase our understanding of the mechanism of action.

Disclaimer/Disclosure:

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1.09.P-Mo057 Defining the EATS Effectome: A Review of Biomarkers Triggered by EATS Model Chemicals

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Model endocrine disrupting chemicals, characterized by their specific modes of action (MoA), are commonly used to study endocrine activity and adversity to support method development within the EATS pathways. However, increasing evidence shows substantial cross-over between endocrine systems, as well as secondary effects non-directly correlated with the endocrine modality of the chemical in question, challenging the assumption that these chemicals act on isolated pathways. Zebrafish exposed to compounds traditionally classified as estrogenic have been shown to influence thyroid histopathology, while thyroid hormone system disrupters have been reported to affect male reproductive development. This illustrates the complexity of endocrine interactions and the need for more integrated evaluations, particularly relevant in zebrafish, a widely used vertebrate model for studying endocrine disruption across multiple levels of biological organization. The aim of this study is to identify and synthesize endocrine-sensitive biomarkers and phenotypic endpoints reported for standard EATS model chemicals in zebrafish. To achieve this, we are conducting a structured literature review combined with analysis of existing Adverse Outcome Pathways (AOPs) associated with these chemicals. The review focuses on mapping commonly observed molecular, cellular, physiological, and developmental effects, identifying biomarkers and endpoints that appear pathway-specific versus those that reflect broader endocrine crosstalk, and highlighting inconsistencies or gaps in current evidence. By integrating available findings with AOP frameworks, this work will provide an overview of how EATS model chemicals manifest across different biological levels in zebrafish and evaluate the extent to which these markers reliably reflect specific or multiple endocrine pathways. This synthesis aims to support the refinement of endocrine biomarkers/endpoints, improve interpretation of results in zebrafish models, and help prioritize areas where additional mechanistic research is needed.

1.09.P-Mo058 Refinement of Fish Embryo Toxicity Test To Assess Ecotoxicological Effects of Nanoparticles

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The Fish Embryo Acute Toxicity (FET) test (OECD 236/2013) with the zebrafish (*Danio rerio*) is designed to determine acute toxicity of a wide range of chemicals on fish embryos' development and survival. This study assesses the contribution of the FET test to the evaluation of potential hazards and toxicity of sonochemically produced Zinc oxide nanoparticles (ZnO NPs), which are widely used as additives in various materials. One of the key benefits is its antimicrobial activity, mainly due to the redox properties of elemental zinc in nano form. Given that the fate, transport, and toxicity of NPs are heavily influenced by their intrinsic physicochemical properties and water chemistry parameters—such as pH, ionic strength (IS), and natural organic matter—we investigated how IS affects the properties and toxicity of ZnO NPs. ZnO nanopowder was dispersed in standard and diluted FET solutions; the latter having different IS, all comparable to that of freshwater. The Dynamic Light Scattering (DLS) technique assessed the NPs' hydrodynamic behaviour and surface charge, while Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES) analysed dissolution. Zebrafish embryos at 3 hours post fertilization (hpf) were exposed to increasing concentrations of freshly prepared ZnO NPs until 96 hpf. Lethality was recorded every 24 hours, hatching was evaluated from 48 hpf, and malformations and morphometric parameters were analysed at 96 hpf.

The results show that the simulated low-IS improved the suspensions' stability by decreasing the NPs' hydrodynamic size compared to standard IS conditions. These physicochemical changes may improve the ZnO NPs bioavailability for developing tissues, increasing the lethal and teratogenic potential of them, evidenced by higher rates of lethality and malformations. Conversely, the inhibition of embryo hatching—the main effect observed under standard test conditions—was partially alleviated under low-IS, as indicated by nearly a tenfold increase in the effect Concentration on Hatching (HC50).

These findings corroborate that the tolerance of zebrafish embryos to various IS media allows refining the FET test to match optimal NPs dispersion to enhance bioavailability and thus increase test sensitivity.

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1.09.P-Mo059 Zebrafish to observe vascular responses associated with preeclampsia risk

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Preeclampsia is a pregnancy disorder characterized by early disturbances in vascular regulation and kidney function. Experimental models capable of detecting these biological features are essential for assessing environmental influences on maternal risk. Zebrafish retain conserved angiogenic and kidney filtration pathways, and PFAS exposure has been epidemiologically linked to increased preeclampsia risk. Based on this evidence, zebrafish were used to examine vascular and kidney-related phenotypes following PFOA and PFOS exposure. Adult zebrafish were exposed to PFAS for 21 days. Fertilized eggs collected on days 7 and 14 were maintained under the same exposure conditions for 96 hours. Larval blood flow and heart rate were assessed using DanioScope, and vessel diameter was quantified with ImageJ to estimate relative vascular pressure. Gene expression related to angiogenesis and kidney filtration was evaluated in larvae and adult tissues. Larvae derived from exposed adults exhibited early functional alterations in vascular activity. Blood flow speed increased at the highest concentration, accompanied by elevated estimated pressure, whereas heart rate was unchanged. Body length decreased at the highest exposure level, indicating developmental stress. In adult gonadal tissue, angiogenesis-related genes showed decreased *vegfa* and increased *eng* expression. Exposed females also exhibited reduced gonadosomatic index, suggesting compromised reproductive condition. These findings demonstrate that zebrafish can detect early vascular and developmental phenotypes relevant to preeclampsia and support their utility for evaluating environmental chemicals that may influence maternal vascular risk.

Disclaimer/Disclosure:

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1.09.P-Mo060 Towards Non-Lethal Ecotoxicology: Validation of a Non-invasive Biomarker Approach for Assessment of Benzo[a]pyrene Exposure in *Salmo trutta*

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Fish populations are experiencing a marked decline due to multiple environmental pressures, particularly chemical pollution. A challenge emerges consisting of finding reliable biomarkers to assess the physiological state of fish exposed to toxic stress using less invasive matrices. In this context, this study aims to apply non-invasive biomarkers, based on the analysis of biomarkers in skin mucus and serum, for comparison with those traditionally analysed in internal organs, to study the effects of pollution on fish. An experiment was therefore conducted on Brown Trout (*Salmo trutta*), exposed to benzo[a]pyrene (BaP), a genotoxic polycyclic aromatic hydrocarbon and common combustion by-product that contaminates aquatic environments and severely compromises fish health. To evaluate the relevance of these non-invasive approaches for monitoring responses associated with oxidative stress, detoxification, and immunity, fish received a single intraperitoneal BaP injection (at concentrations of 0.5 mg/kg or 10 mg/kg). A panel of biomarkers, including Ethoxyresorufin-O-deethylase (EROD), Glutathione S-Transferase (GST), Superoxide Dismutase (SOD), Carboxylesterase (CE), and bactericidal activity was subsequently analysed in the liver and mucus at different exposure times: 72 hours and 7 days after injection. The results confirmed significant BaP bioaccumulation in the liver and a strong hepatic EROD induction at 7 days, thereby validating the accumulation and the activation of Phase I detoxification pathways. Furthermore, BaP exposure increased the CE and SOD activity in the mucus, suggesting that oxidative stress can be detected via this non-invasive matrix. Nevertheless, other biomarkers, such as GST and bactericidal activity, showed no significant modulation. These observations reveal that non-invasive matrices reflect key physiological changes, often with a divergent temporal dynamic compared to the liver. These results confirm the relevance of BaP as a contaminant model and highlight the value of mucus as non-invasive matrix. They represent a promising tool for assessing the effects of pollution on fish, allowing relevant physiological information to be obtained without resorting to invasive methods.

1.09.P-Mo061 Reproductive toxicity of tributyl phosphatate (TBP) and its underlying mechanisms in zebrafish and fathead minnows

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Due to the phase-out of polybrominated diphenyl ethers (PBDEs) in the global market, the use of organophosphate esters (OPEs) has increased as an alternative flame retardant. Among OPEs, tributyl phosphatate (TBP) is widely used in various applications, including building materials, electronics, textiles, and furniture, to prevent the ignition and spread of fires and protect human life and property. Due to its lipophilicity, TBP can persist in the environment and bioaccumulate, raising potential health concerns for both humans and aquatic organisms. Experimental studies have shown the sex hormone and neuroendocrine-disrupting effects of OPEs; however, toxicological information on TBP is largely limited. In the present study, we aimed to investigate the reproductive toxicity of TBP and its underlying mechanisms, with a particular focus on gonadal lipid metabolism and sex steroid biosynthesis, using two model fish species: the fathead minnow (*Pimephales promelas*) and the zebrafish (*Danio rerio*). Following OECD Test Guideline 229, adult breeding pairs of both fathead minnows (FHM) and zebrafish (ZF) were exposed to TBP at nominal concentrations of 0, 0.03, 0.3, and 3 mg/L for 21 days. Fecundity and gene expression changes related to lipid metabolism and steroidogenesis were measured. The number of cumulative eggs produced significantly decreased in a concentration-dependent manner in both adult FHM and ZF following 21-day exposure to TBP. This reproductive decline could be explained by the altered gene expression of gonadal steroidogenesis, i.e., *3bhsd*, *17bhsd*, *11b2hsd*, *cyp17*, and/or *cyp19a* in the gonads of both adult FHM and ZF. Moreover, the gene expression of *ppar?*, *srebp1*, and/or *srebp2*, which are critical for fatty acid and glucose metabolism, was disrupted in the gonads in both species, suggesting a metabolic disruption could partly explain the reduced fecundity. Overall, our results indicate that exposure to TBP may impair reproductive function by disrupting steroidogenesis and gonadal lipid metabolism in both FHM and ZF. Considering the environmental persistence and bioavailability of TBP, further research is needed on its long-term and transgenerational effects to understand its ecological risk.

1.09.P-Mo062 A Mechanistic approach to regulatory compliant Quantitative Structure-Activity Relationship modelling for chronic fish

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Of all the studies in environmental toxicology, the chronic fish test is probably the hardest to replace and in doing so, would save the highest number of vertebrate animals.

QSARs have long been seen as “screening” tools with limited capacity to directly replace experimental studies let alone chronic ones. Nevertheless, the release by the OECD of the QAF (5 principles for model validation and 4 principles for prediction validation) reduces uncertainty in predictions. The QAF aided in the production of far more accurate models that can serve as stand-alone replacements to experimental guideline studies.

High accuracy QSARs have been created combining two approaches:

1) mechanisms of toxic action (MechoA), similar to Molecular Initiating Events (MIE) are inextricably linked to two keystones: on one side, structural features and on the other, biological matrix interactions. More than one structural group can have the same mechanism of action (thus, the same toxicological impact).

2) The use of the chemical activity approach relating the external metric “water solubility” to the toxicity endpoint (EC10) using only data that was rigorously validated internally to create “High Accuracy QSARs”. These models, in the KREATiS iSafeRat® model suite may thus be better termed Quantitative Mechanism-Activity Relationships or “QMARS”.

Original fears that chronic QSARs were not possible due to unaccounted for metabolic impact on the test substance in long term studies were rejected as such metabolism was inherently included in the training set data. At this stage the iSafeRat® Platform boasts only 4 QSARs for fish chronic predictions. However, as these are related to 4 MechoA sub-Classes, and each sub-class contains multiple structural groups, the diversity of structures for which accurate predictions of chronic fish toxicity can be made is extremely high, covering thousands of REACH registered substances for which chronic data are currently absent.

While in silico methods cannot replace all chemicals (e.g. nanoparticles, UVCBs where potentially bioavailable constituents are unknown), the use of a reliable model like the QMARS of the iSafeRat® platform can serve as a stand-alone replacement or as part of a weight of evidence approach with confidence when falling within the applicability domain of the model, significantly reducing testing for the majority of chemical substances and ultimately for biocides, agrochemicals and pharmaceuticals as the science progresses.

1.09.P-Mo063 Assessment of Macrophage Counts in Tg(mpeg1) Zebrafish Following PFAS Exposure

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Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern increasingly found in the environment and human exposure pathways. Many PFAS can cross the placental, and blood brain barriers, resulting in developmental exposure. Macrophages are central elements of innate immunity, contributing to both homeostasis and immunomodulation. Microglia are resident macrophages in the Central Nervous System that play a key role in neurodevelopment, immune responses, synaptogenesis, and the maintenance of neural circuits. Disruptions in their function during critical developmental windows can impact both immune and neural outcomes underscoring the need for an understanding of their potential impact on the developing immune and nervous systems during chemical exposure. To address this gap, we assessed the impact of PFAS exposure on macrophage count and morphology in the whole body and head region using the (mpeg1:mCherry) zebrafish, which express an mCherry fluorophore under the control of the macrophage-specific mpeg1 promoter. Exposure levels were based on concentrations previously measured in the blood of Scandinavian populations (1x or 1000x the human blood concentration (HBC)). Zebrafish embryos were exposed from 0-4 days post fertilization (dpf) to 1000x HBC of either PFOA, PFHxS, PFNA, PFDA, PFUnDA, or 1x HBC of either PFOS or a ratio-based mixture of all six PFAS. The potential effect on macrophage numbers was compared to the solvent control (0.1% dimethyl sulfoxide) and to dextran sodium sulfate (DSS, 0.18%), a potential positive control which was previously shown to increase macrophage numbers. To quantify macrophages and evaluate morphology, embryos were anesthetized and embedded in agarose. High-Content Images were acquired as z-stacks over three tiles covering the entire larva using an ImageXpress Confocal HT AI system, enabling automated quantification of macrophages with a bespoke Python-based analysis pipeline. Analysis is underway and will quantify total body macrophages, and head region macrophages in ratio to whole body counts after exposure. Preliminary results show that exposure to 0.18% DSS increased whole body macrophage numbers relative to the solvent control, and a trend towards lower numbers following PFNA exposure (1000x HBC). Taken together, this approach will reveal whether exposure to single PFAS or a representative mixture results in reduced macrophage numbers, potentially indicative of dampened immune function.

1.09.P-Mo064 Use of RTL W1 Liver Cell Line in a Testing Platform to Monitor

Biotransformation/Degradation and for Mode of Action Studies for Nanomaterials; A Case study using Boron nitride (BN)

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The RTL W1 cell line from rainbow trout liver holds the potential to be used as a test system for long term studies on biotransformation/degradation because it has relevant metabolic capacities, and one can assess the extent to which a potential toxicant activates specific pathways or induces key metabolic enzymes in a bid to try to identify the real mode of action. The importance of performing longer term experiments in such cellular models lies in the fact that materials such as nanomaterials can change (transform/degrade) over time within these biological systems. Depending on the extent of this transformation/degradation, effects could either be driven by the pristine material “per se”, the transformed material or indeed any degradation products. This information is important for a complete understanding of the hazard, to determine what is driving effects and the ultimate biological fate of these materials. In this study the RTL W1 cell line was used to investigate the role of the aryl hydrocarbon receptor (AhR)/cytochrome P450 1A (CYP1A) biotransformation pathway activation in the cellular processing of nanotubes and nanosheets of boron nitride (BN) following 24h, 72h and 7 days exposure. After 7 days the nanosheets remained intact in cellular vesicles with no obvious transformation or degradation, while in cells exposed to nanotubes there was evidence of membrane piercing and much smaller transformed nanotubes in the cytoplasm. The AhR dependent CYP1A-catalyzed 7-ethoxyresorufin-O-deethylase (EROD) activity assay revealed increases in activity and increased gene expression of *ahr* and *cyp1a1* were measured in RTL W1 cells following exposure to the BN nanotubes, implicating activation of the AhR/CYP1A signaling pathway in the cellular processing of this material. On the other hand BN nanosheets did not increase EROD activity nor gene expression levels at any exposure time. Increased reactive oxygen species (ROS) were also measured following BN material exposure suggesting oxidative processes are also involved in the mode of action. This investigation using the RTL W1 liver cell line is the first to confirm the activation of this important biotransformation pathway by nanotubes of BN (or its degradation products). Such a platform is useful for nanomaterial biodegradation/transformation testing and to identify potential AhR/CYP1A pathway activation in the mode of action for other aromatic like nanostructures or degradation products.

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1.09.P-Mo065 Rainbow Trout Alevin (RTA) Assay: Comparisons of transcriptomic points of departure with fish 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; here, we focus on the ability of this test to help predict fish chronic toxicity. 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). 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. For most chemicals, putative LC50 values were obtained, and these tend to correlate with 96-hr LC50 values (fish acute toxicity). Sequencing data have been obtained for 33 chemicals. Bioinformatics analysis of these data using ExpressAnalyst shows that the number of gene benchmark doses ranges from 113 to 2118 across the dataset, with an average of 733 per chemical. For

each chemical, a tPOD (either mode, 10th percentile, or 20th gene) was calculable, with tPOD mode values ranging from 0.008 to 1,500 mg/L. Currently we have fish chronic toxicity data for 15 chemicals, with a statistical association between the tPODs data obtained here (i.e., 24 hour assay with fish embryos) and fish chronic toxicity data (i.e., whole animal studies that are at least 21 days in duration). 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, expand the fish acute/chronic data, and continue discussions between academic and government partners.

1.10 From Mechanistic Insights and Innovation to Regulation: Integrating Behavioural Endpoints, AOPs and NAMs into Next Generation Environmental Risk Assessment

1.10.T-01 Every data point matters – maximising value for regulatory toxicology

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When New Approach Methodologies (NAMs) achieve the quality seal of the Organisation for Economic Co-operation and Development's (OECD) test guideline (TG) programme, they move a crucial step closer to regulatory use. Indeed, regulatory agencies, such as the European Chemical Agency, expect data submissions to build on OECD TG compliant performance, including following Good Laboratory Practice, which usually requires a Contract Research Organisation environment.

However, academic laboratories also frequently benefit and apply OECD TG and are free to publish the achieved results. Such academically derived data sets can comprise an invaluable resource not only to the scientific community but to regulatory toxicologists as well. This is especially true for newly developed OECD TG, where rapidly growing data sets can promote confidence building, enhance knowledge about the applicability domain, and identify needs for further developments. Yet, to count in a regulatory science context, these data must be reported in a way that key elements and validity criteria can be traced.

As developers and distributors of knowledge on OECD TG that aim to substitute toxicity testing using fish, we are continuously screening academic reports and publications – here with regards to OECD TG249 (Fish Cell Line Acute Toxicity - The RTgill-W1 cell line assay) as example. We noted two principal digressions in the open literature that create hurdles for application in the regulatory space: (i) incomplete reporting of essential experimental details; (ii) alterations to the procedure that make it difficult to judge whether the changes impact the predictive ability of the assay as a surrogate for the acute fish LC50 as intended. These findings motivated us to create a template that encompasses details with respect to test substance information, in vitro assay performance and validity criteria. We envision this template to serve several important causes, including structured reporting and facilitated entry into databases, such as the Fish Acute Cell Toxicity database (FACTdb).

Taken together, academic laboratories can contribute a great deal toward the mainstreaming of OECD TG featuring NAMs. When following the TG exactly as developed whenever possible, paying particular attention to key experimental details and criteria for validity, and reporting them accordingly, the data can be of direct and instrumental use for regulatory science.

Disclaimer/Disclosure:

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1.10.T-02 Mode of Action Assessment in *Raphidocelis subcapitata* by Complementing the OECD 201 Test with Transcriptomics

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The growing number of chemicals worldwide increases the need for risk assessment and investigation of their environmental effects on organisms in order to protect the earth's ecosystems. For this purpose, it is very helpful to understand the effects of substances at the molecular level. The combination of ecotoxicological tests and transcriptome analysis makes it possible to identify and understand the modes of action (MoA) of substances in non-target organisms at the gene expression level. The aim of this study was to combine the workflow of the Algal growth inhibition test (OECD Test Guideline (TG) 201) with transcriptome analysis to gain insight into the MoA of substances in the freshwater microalga *Raphidocelis subcapitata* (R. subcapitata).

The toxicity test in R. subcapitata, according to OECD TG 201, was followed by transcriptomics and provided insights into the molecular effects of two substances, the photosynthesis-inhibiting herbicide bentazone and the zinc oxide nanoparticle NM-110, which is widely used in products such as sunscreens for its UV-absorbing properties. Several range finding tests were performed to determine low effective concentrations (e.g. EC5 and EC20). Main tests based on these concentrations and subsequent RNA extraction using a newly established RNA extraction protocol for R. subcapitata formed the basis for the subsequent gene expression analysis. Combined with an overrepresentation analysis, these analyses showed that exposure to low effective concentrations of each substance (Bentazone: EC5=9.96 mg/L, EC20=24.2 mg/L; NM-110: EC5=0.033 mg/L, EC20=0.041 mg/L) resulted in a large number of differentially expressed genes compared to the unexposed control. The molecular effects observed could be partially related to the MoA described in the literature for each substance. Comparative analysis indicated different MoA for the two substances. However, both substances shared a common link to the generation of reactive oxygen species and thus also to oxidative stress.

In conclusion, this study could positively contribute to a better understanding of the molecular effects and MoA of the two substances, bentazone and the zinc oxide nanoparticle NM-110, in R. subcapitata. The development of an RNA extraction protocol for the alga was fundamental for this. The results of this study also provide a basis for further screening approaches with other chemicals.

1.10.T-03 Sweet but Toxic? An Ecotoxicological Hazard Assessment of the Artificial Sweetener Acesulfame using Toxicogenomics

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Acesulfame (ACE) is widely used as an artificial sweetener, primarily in food and beverages. Due to its chemical stability and water solubility, it remains unmetabolized and is not fully removed during wastewater treatment, resulting in environmental persistence and mobility in aquatic systems. Despite recognition as an emerging contaminant for years, a comprehensive environmental risk assessment is still lacking, as regulation for food additives does not require it. Aligned with the goals set by PARC, this project investigates the ecotoxicological effects of ACE via toxicogenomics. Three aquatic model organisms of different trophic levels were exposed to analytically verified concentrations ranging from environmentally relevant levels (10 µg/L) to the upper limit used in substance evaluation (100 mg/L). Following a tiered testing approach in line with next generation risk assessment, standardized acute toxicity tests (OECD) were combined with transcriptomics. Statistically significant changes in gene expression were observed in all organisms. Functional analyses indicated oxidative stress as the dominant effect in zebrafish, while lemna exhibited several stress and defense responses. Notably, environmentally relevant concentrations of ACE affected neuroendocrine pathways in daphnids and cluster analysis showed transcriptomic similarity to pyriproxyfen, a known developmental disruptor. To connect molecular findings with regulatory endpoints, a chronic toxicity test with daphnia at low concentrations is planned. As juvenile hormone mimics typically impair moulting in daphnia, population-level effects may be expected. In prior higher-concentration tests, systemic effects might have masked endocrine disruptions.

In summary, this study underscores that ACE induces stress-related transcriptional changes across aquatic model organisms and reveals early signs of neuroendocrine disruption in daphnids. For a food additive not previously considered in environmental risk assessments, these findings are concerning. The results exemplify how OMICs approaches can advance hazard assessment by sensitively identifying sublethal effects with potential long-term consequences. Building on the daphnia results, transcriptomics indicate that chronic testing at environmentally relevant concentrations is warranted, thereby linking molecular and population-level effects and promoting more efficient, animal-friendly hazard assessment.

Disclaimer/Disclosure:

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1.10.T-04 Comparative Behavioral Screening of Environmental Chemicals in *Daphnia magna* and *Drosophila melanogaster*

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The increasing presence of neuroactive chemicals in the environment poses significant risks to the developing nervous systems of humans and wildlife. Behavioral endpoints represent early and sensitive indicators of neurotoxicity and ecological impairment, yet they remain insufficiently integrated into regulatory ecotoxicology frameworks. To advance the application of behavior within New Approach Methodologies (NAMs), we performed a cross-species behavioral screen of 200 environmentally relevant chemicals using *Daphnia magna* and *Drosophila melanogaster* as complementary model organisms.

High-throughput behavior tracking platforms were used to quantify locomotor activity under sublethal exposure conditions across three concentrations per chemical. A machine learning-based algorithm was developed to benchmark and extract quantitative behavioral features. Behavioral signatures demonstrated strong reproducibility across exposure levels and experimental replicates. Chemical clustering based on locomotor phenotypes revealed organophosphate pesticides as consistently neuroactive across both species, while additional compounds produced species-specific behavioral disruptions, reflecting differences in neurophysiology and exposure susceptibility.

These results highlight the feasibility and value of behavior-based NAMs for comparative neurotoxicity screening and chemical prioritization, and support the broader use of behavioral endpoints in ecologically relevant and mechanism-informed hazard assessment.

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1.10.P From Mechanistic Insights and Innovation to Regulation: Integrating Behavioural Endpoints, AOPs and NAMs into Next Generation Environmental Risk Assessment

1.10.P-We012 EthoCRED: A Framework to Guide Reporting and Evaluation of the Relevance and Reliability of Behavioural Ecotoxicity Studies

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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.

1.10.P-We013 Scaling Up Biomonitoring: Eukaryotic Community-Wide 18S Profiling Enables to Identify Priority Taxa Across Upstream–Downstream Pollution Gradients

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The Water Framework Directive, among others, requires the monitoring of biodiversity or ecosystem structure. Conventional environmental biodiversity monitoring relies on a few indicator groups of organisms with a proven history of sensitivity to contamination (e.g., diatoms). However, the targeted species only provide an incomplete picture of the community's full taxonomical range and its diverse contaminant response profile. Additionally, biodiversity indices are routinely calculated based on monitoring data because they condense complex community structure into simple values. However, biodiversity indices can mask important ecological features such as taxonomic turnover, functional replacement, and stressor-driven shifts, all essential to understand the effects of contaminants. To complement these conventional metrics, we applied eukaryotic 18S metabarcoding as a holistic screening tool to detect community-wide responses to local metal pollution along a stream (Grote Nete - GN, Belgium). Baskets containing five stones were installed at each site during 8 weeks for later biofilm collection. Biofilm DNA was extracted for metabarcoding sequencing (18S rRNA) and further taxonomic assignment using SILVA 138. We coupled overall richness and Shannon diversity (ANOVA)—considering all species without phylum-level distinction—with phylum-level diversity metrics and ALDEx2-based differential abundance testing to assess site-level contrasts between upstream and downstream locations. Among the 48 phyla detected in GN, Shannon diversity significantly differed for 3 groups (e.g., Ascomycota), and richness for 3 others (e.g., Porifera)—post hoc Tukey, indicating changes in the internal structure of these phyla—such as shifts in taxa evenness. While ALDEx2 paired-site comparisons revealed significant upstream–downstream shifts for more than 10 groups (e.g., Blastocladiomycota), reflecting changes in the dominance of these phyla, as pollution-driven selection and competitive displacement cause some groups to become more prevalent while others decline.

Our findings reveal that 18S rRNA gene metabarcoding survey rapidly and effectively diagnoses eukaryotic groups most affected by metal pollution and efficiently prioritizes group-specific assays for ongoing targeted monitoring (e.g., ITS genetic region for Blastocladiomycota), complementing regulatory programs and improving early detection of pollution-driven ecological change.

1.10.P-We014 Aligning the REACH Revision with the EU Roadmap to Phase Out Animal Testing for Stronger Environmental Protection

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The REACH revision marks a critical moment to reshape chemical safety assessment in the European Union. The EU has committed to ending animal testing for chemical safety assessments – a pledge driven by the Chemicals Strategy for Sustainability (CSS), the European Parliament's 2021 resolution concerning the CSS, and the Commission Roadmap towards phasing out animal testing for chemical safety assessments in response to the EU Citizens' Initiative "Save Cruelty Free Cosmetics – Commit to a Europe Without Animal Testing". To translate this commitment into practice, the revised legislation must establish non-animal approaches as the standard for chemical safety assessments.

Non-animal approaches based on mechanisms of action can assess risks across a wider range of species, offer greater ecological relevance and support more timely and informed regulatory assessments. These methods help overcome the well-established limitations of tests on animals and hold the promise of equal or higher environmental protection while reducing reliance on animal testing.

To deliver this shift, key reforms are required to modernise the governance of chemical safety assessment. We propose introducing the concept of information objectives by defining information on intrinsic properties of substances that must be generated and assessed against clearly defined objectives that serve the overarching protection goal, rather than relying on standard information requirements based on prescribed test mandates. Building on this foundation, the revision should strengthen the legal requirement that tests on animals be used only as a last resort. In addition, we recommend that registration, testing proposals, and compliance checks systematically prioritise non-animal approaches, thereby enhancing transparency, preventing redundant testing, and enabling the wider use of existing evidence. The revision should also advance the development, implementation and regulatory integration of innovative environmental safety methods and be supported by an independent expert committee to ensure consistent, science-based evaluation across all processes.

Together, these reforms would future-proof environmental assessments and improve regulatory consistency, enabling the European Union to honour its commitment to ending animal testing while advancing scientific innovation and strengthening environmental protection.

1.10.P-We015 Immunomodulatory In-vitro Effects of Realistic Environmental Pollutant Mixtures on Innate Immunity

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Humans are continuously exposed to complex mixtures of chemicals, including endocrine-disrupting compounds (EDCs), which are increasingly associated with the growing incidence of non-communicable diseases. The immune system, which is interconnected with the endocrine system, plays a fundamental role in maintaining overall health, yet remains an overlooked target in toxicology. Due to the complexity of immune cell interactions, data on immunotoxic effects are still limited, and current OECD test guidelines are sparse. In vitro bioassays offer promising approaches for evaluating immune-related health endpoints, including inflammation, antiviral response, immune cell proliferation and general stress responses, that reflect overall innate immune system function. Essential transcription factors and receptors covering those health endpoints are NF- κ B (nuclear factor kappa B), IRF (interferon regulatory factor), AP-1 (activator protein 1), AhR (aryl hydrocarbon receptor) and PPAR γ (peroxisome proliferator-activated receptor gamma). The inhibition of such signaling pathways is rarely evaluated in current screening strategies. We tested representative mixtures of pan-European chemical exposure (including PFAS, PAHS, Phenols, Pesticides and Phthalates) in a test battery covering these endpoints in agonism and antagonism mode. We observed antagonistic effects of the mixtures and their individual components at realistic concentration ratios, exemplify the interference of NF- κ B (four out of six tested mixtures), IRF (four out of six tested mixtures), and PPAR γ (2 out of six mixtures). Our results highlight the applicability of in vitro bioassays to screen realistic mixtures of chemicals for the capability to disrupt health-relevant molecular processes and to explore how chemicals act together in mixtures.

1.10.P-We016 Developmental Neurotoxicity mediated through Endocrine Disruption in Fish: A Systematic Review

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Developmental neurotoxicity (DNT) is defined as “chemical-induced interference with normal development of the nervous system, which, if uncompensated, may lead to adverse effects on nervous system structure and/or function” (OECD). Endocrine-disrupting chemicals (EDCs) can interfere with hormonal signaling pathways that are critical for brain development, such as thyroid and estrogen systems in vertebrates. Disruption during sensitive developmental windows may lead to long-term behavioral and physiological effects. However, the mechanistic interaction between endocrine disruption (ED) and DNT remain unclear. Regulatory agencies emphasize the need for Adverse Outcome Pathway (AOP)-based frameworks to assess DNT, as these approaches integrate mechanistic evidence across biological levels and support predictive, regulatory-relevant testing strategies.

This systematic review aims to identify knowledge gaps and evaluate the credibility of existing evidence and approaches for studying endocrine disruption-mediated DNT in vertebrate fish. This will be achieved by using a systematic review approach to evaluate factors including study design parameters, chemical identity, exposure conditions and neurodevelopmental endpoints assessed. Preliminary insights suggest that most studies focus on transcriptional analysis and behavioral endpoints, while morphological and molecular endpoints are less investigated. This implies a knowledge gap regarding mode of actions (MoA) on key events placed between molecular initiating events (MIEs) and adverse outcomes, as intended in the Adverse Outcome Pathway (AOP) framework. This highlights the need for both transcriptional analysis, molecular markers, morphology and behavioral endpoints, to improve mechanistic understanding and ensure relevance for regulatory decision-making.

Our review provides a foundation for development of robust testing strategies, and prioritizing research to address critical uncertainties in DNT assessment in fish mediated through endocrine pathways.

1.10.P-We017 C. elegans as a Model Organism to Complement the OECD Developmental Neurotoxicity In Vitro Battery

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Only a small fraction of chemicals on the market have been fully evaluated for developmental neurotoxicity (DNT). Current *in vivo* test guidelines (OECD TG 426 and TG 443) are not mandatory in international regulatory frameworks and remain costly, time-consuming, and often insensitive to subtle neurodevelopmental effects. As a result, there are large data gaps for industrial and agricultural chemicals, underscoring the need for faster and more predictive DNT screening approaches.

In 2023, the OECD published guidance on interpretation of data from the *in vitro* battery (IVB), a set of 17 cell-based assays that target key neurodevelopmental processes. While the IVB represents major progress toward NAM-based DNT testing, important endpoints remain insufficiently covered, including whole-organism responses, learning and behaviour, metabolic and endocrine interactions, and systemic compensatory mechanisms. Integrating a whole-organism model with the IVB could therefore strengthen mechanistic interpretation and improve detection of DNT hazards.

The nematode *C. elegans* offers a promising complementary model due to its conserved neurodevelopmental pathways, well-characterised nervous system, genetic tractability, and suitability for rapid, high-throughput behavioural assessment. An image-based platform capable of detecting changes in behaviour may identify whole-organism level DNT effects not measurable by cell-based assays, therefore providing more comprehensive screening that supports efforts to advance NAMs in chemical safety assessment.

This project will test a subset of the positive and negative control chemicals used in the IVB, enabling nematode behavioural endpoints to be benchmarked against existing IVB data. This comparison is essential for validating the behavioural platform and demonstrating how whole-organism responses may complement DNT-relevant endpoints not captured by the current IVB. Furthermore, incorporating strain variation in this platform will better reflect genetic diversity and support the assessment of population-level differences in susceptibility, while also helping refine DNT-relevant AOPs. Together, these advances position *C. elegans* as a valuable whole-organism tool to address current gaps in the OECD DNT IVB and to accelerate the transition toward predictive, ethical, and efficient DNT testing strategies.

1.10.P-We018 Development of an Automatic High-Throughput Behavioural Phenomics Platform for *Daphnia magna* Toxicity Screening

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Daphnia species, particularly *Daphnia magna*, are widely used sentinel organisms in freshwater ecotoxicology due to their ecological relevance, short generation time, clonal reproduction, and sensitivity to a broad range of chemical stressors. Their well-characterised physiology and transparent bodies make them ideally suited for mechanistic and behavioural studies. Although behavioural responses in *Daphnia* provide rapid, sensitive, and non-invasive indicators of sublethal stress, behavioural endpoints remain underutilised in regulatory frameworks, which still rely heavily on binary immobilisation outcomes and other low-resolution metrics.

To address this gap, we developed and validated a high-throughput behavioural phenotyping platform using *Daphnia magna*, designed to support reproducible and scalable chemical screening across multiple strains and treatments. We established a ten, 24-well plate assay system integrated with environmental stabilisation measures and automated video tracking. The platform is designed for automated acquisition of behavioural data including immobilisation, speed and frequency of locomotion, and spatial positioning, using behavioural tracking software and analytical workflows. Multiple clonal lines of *Daphnia magna* from distinct environments are being incorporated to allow assessment of genotype-dependent behavioural variability both under controlled conditions and during stress response.

We aim to establish whether the platform can generate consistent behavioural profiles over 48hr exposures and whether behavioural endpoints offer earlier or more nuanced detection of chemical stress than conventional acute exposure endpoints. Future integration with transcriptomic profiling will enable mechanistic interpretation of behavioural phenotypes in relation to pathways and processes of toxicological relevance.

Overall, the vision system is intended to accelerate next-generation ecotoxicological assessment by improving sensitivity, mechanistic relevance, and throughput, with the longer-term goal of supporting chemical screening and risk assessment across diverse *Daphnia* genotypes.

1.10.P-We019 Evaluation of Patulin-Induced Neurotoxicity: Effects on Neurotransmitters, Neural Damage, Behavior, and Mechanisms in *Caenorhabditis elegans*

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Patulin (PAT), a mycotoxin mainly produced by *Penicillium expansum*, is commonly found in moldy fruits, vegetables, and grains, especially apples and apple-based products. Due to its heat and acid stability, humans are frequently exposed through fruit juices and infant foods. PAT is known to cause multiple toxic effects, including acute nausea, vomiting, and gastrointestinal discomfort, and long-term exposure may damage the kidney, liver, and immune system. However, studies on its potential neurotoxicity are relatively limited, and the underlying mechanisms remain unclear. Therefore, this study uses *Caenorhabditis elegans* to determine whether PAT induces neurotoxicity and how it affects neurotransmitters and physiological behaviors.

We evaluated developmental, locomotion, and feeding endpoints under various PAT concentrations. The results showed that PAT significantly inhibited worm growth at 50-90 μ M, reduced head thrashing at 0.1-90 μ M, and markedly decreased pharyngeal pumping at 10-90 μ M.

In *C. elegans*, head thrashing is controlled by head neurons involving serotonin and dopamine, and pharyngeal pumping is primarily regulated by serotonin. To assess serotonergic function, we performed an egg-laying assay, reflecting serotonin- and neuropeptide-regulated vulval muscle contraction, and found that PAT significantly reduced egg laying at 10-90 μ M.

Overall, PAT disrupts head thrashing, pharyngeal pumping, and egg laying, suggesting a specific impairment of serotonergic neurons. We will further explore the molecular mechanisms linking neurons and neurotransmitters to define the in vivo neurotoxic profile of PAT.

1.10.P-We020 Cocaine Pollution Alters the Movement and Space Use of Atlantic Salmon (*Salmo salar*) in a Large Natural Lake

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Cocaine and its metabolites are increasingly being detected in aquatic environments worldwide. While previous research has demonstrated that environmental concentrations of these neuroactive substances can affect brain function, behaviour, and energetic traits in various species, this research has exclusively been conducted under simplified laboratory conditions that fail to capture real-world complexity. As a result, how cocaine pollution affects animal behaviour and movement in the wild is not known. Here, we combine novel slow-release chemical implants with acoustic telemetry tracking to reveal how environmentally realistic levels of cocaine and its main metabolite benzoylecgonine affect movement and space use of Atlantic salmon (*Salmo salar*) smolts in a large natural lake (Lake Vättern, Sweden). Both cocaine and benzoylecgonine exposure increased weekly movement rates of fish in the wild, with this effect being particularly pronounced in the benzoylecgonine treatment group. Further, benzoylecgonine increased dispersal and large-scale space use within the lake relative to controls. Our results demonstrate that environmental levels of illicit substances like cocaine and its metabolites can modify animal behaviour and space use in the wild, with potential consequences for species and community ecology.

1.10.P-We021 From gasoline to green fuels: Uncovering neurotoxic effects in developing zebrafish

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Climate-neutral mobility is becoming increasingly important as the European Union plans to ban new fossil-fuel combustion vehicles by 2035. Yet millions of existing combustion vehicles will remain in use, making bio-hybrid fuels (produced from renewable feedstock's, CO₂, and green hydrogen) a promising transitional option. Despite their sustainability potential, their toxicological profiles remain insufficiently understood. Conventional fossil fuels, such as gasoline, are known to cause adverse health effects, especially negative neurological outcomes including headaches, hyperactivity, and even sedation. Given this evidence, it is important to assess whether bio-hybrid fuels may similarly affect the nervous system during production, transport, use, and accidental release.

Here, we evaluated neurodevelopmental effects of the bio-hybrid fuel blend KEAA in zebrafish embryos (*Danio rerio*), alongside gasoline as a reference fuel and caffeine as a neurotoxic positive control. Neurobehavioral endpoints (spontaneous coiling, touch-evoked escape response, and light/dark locomotor activity) were assessed up to 120 hpf and complemented by morphological measurements.

KEAA induced clear, concentration-dependent neurobehavioral alterations, including early coiling deficits and reduced escape responses at low concentrations. Caffeine did not affect coiling, but reduced escape behavior at later stages. Gasoline water-accommodated fractions similarly impaired coiling and caused the strongest reduction in escape responses among the tested substances. KEAA additionally produced morphological alterations, most notably reduced body length and eye size, whereas caffeine did not elicit strong morphological changes. Gasoline-related morphological effects are currently under evaluation.

This work underscores the importance of integrating apical, behavior-based endpoints into early-stage safety assessment of emerging alternative combustion fuels using the zebrafish model. This approach is particularly valuable when evaluating complex or previously uncharacterized mixtures, as behavioral readouts provide sensitive indicators of subtle or unexpected effects that can subsequently be explored through more mechanistic methods such as transcriptomics or proteomics.

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1.10.P-We022 Towards animal-free environmental safety assessment for petroleum UVCB: challenges and adaptations within the EU Roadmap to phase out animal testing

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The European Commission (EU) has launched a Roadmap to phase out animal testing across 15 chemical legislations, setting short-, mid-, and long-term milestones (EC 2023, EC 2024) to advance with the application of New Approach Methodologies (NAMs) to ecological risk assessments without animal testing. Although NAMs offer significant potential in this paradigm shift, their applicability domain must be carefully considered, particularly for complex chemistries such as petroleum substances, known as substances of Unknown or Variable Composition, Complex Reaction Products, and Biological Materials (UVCBs). The petroleum UVCB substances pose specific challenges for NAMs implementation, including undefined specific molecular structures, compositional variability, and the presence of a very large number of constituents, making them difficult to be fully characterized. Their analytical and inherent variable complexity, including low solubility in aqueous solution, high hydrophobicity and volatility, combined with their nature as non-intentional mixtures, difficult the use of some in vitro dosing and modelling strategies.

This poster will identify key adaptations required to enable a feasible transition for petroleum substances within the EU Roadmap to phase out animal testing, including: (i) reducing animal testing through robust read-across approaches; (ii) achieving regulatory acceptance of existing NAMs for testing UVCB; (iii) advancing in vitro dosing strategies for complex substances; (iv) integrating legacy in vivo data to inform mechanistic models; and (v) tailoring New Generation Risk Assessment (NGRA) frameworks to address UVCB-specific challenges. These adaptations will be structured with the three key elements of the EU Roadmap including short-term priorities focus on compliance and minimizing animal use; mid-term goals emphasize in vitro and in silico integration; and long-term objectives aim for full replacement of animal testing through adapted NGRA paradigms.

By addressing these scientific and regulatory hurdles, the chemical industry can accelerate the transition towards an animal-free environmental safety assessment, ensuring the use of robust scientific methods to protect aquatic ecosystems.

Key words: EU Roadmap, New Approach Methodologies, NAMS, petrochemical substances, UVCBs

1.10.P-We023 Addressing a Regulatory Blind Spot in Traditional Ecotoxicity Methods

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Environmental risk assessment still focuses on traditional regulatory endpoints (e.g., mortality, reproduction), while pollutant effects on host-associated microbiomes remain neglected. Thus, examining antibiotic-induced microbiome shifts in *Daphnia* sp. is not only relevant to organismal fitness but also a warning for the promotion of antibiotic-resistant bacteria (ARBs) and the spread of antibiotic resistance genes (ARGs) in the environment. Our purpose was to test whether fluoroquinolone antibiotics reshape the *Daphnia magna* microbiome in ways consistent with selection, even when effects on traditional *Daphnia* endpoints are minimal.

A chronic 21-day *D. magna* reproduction test was conducted following the OECD guideline 211 with

ciprofloxacin (CIP), ofloxacin (OFX), and new fluoroquinolones designed to have higher degradability through hydrolysis (e.g., ciprofloxacin-hemi). Bioassay concentrations ranged from 10 to 1,049 µg L⁻¹ of each antibiotic. After exposure, pooled samples (5 organisms per antibiotic concentration) were flash frozen and kept at -80 °C until DNA extraction. Full-length 16S rRNA genes were sequenced (27F/1492R, PacBio); reads were processed in QIIME 2 with DADA2 to obtain ASVs. R software was employed to identify differences between survival, reproduction, as well as to compute diversity and microbiome compositional profiles. Based on standard regulatory observations, no significant toxic effects regarding survival or reproduction were identified. For sensitive/tolerant taxa, we hypothesize that there is concentration-dependent microbiome restructuring with selection of resistant bacteria.

Microbiome-based research could complement traditional regulatory endpoints, capturing host-relevant mechanisms. This study employs molecular endpoints for next-generation environmental risk assessment, revealing how host-linked microbiome indicators can be integrated into decision-making by bridging innovation into the regulatory practice.

1.10.P-We024 Comparative Dietary Analysis in Small Mammals: Fecal vs. Stomach Content eDNA Metabarcoding in Herbivores and Insectivores

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Dietary analysis is essential in European pesticide risk assessment for wild birds and mammals, as species-specific diets and foraging behaviors directly affect exposure estimates. Traditionally, such analyses relied on invasive stomach content examinations or visual inspection of fecal samples, both limited by methodological constraints. Environmental DNA (eDNA) metabarcoding offers a non-invasive alternative, enabling simultaneous host and diet identification from fecal samples. This study compares fecal-based eDNA metabarcoding for host identification to dietary profiles from stomach content samples in two model organisms: *Microtus arvalis* (herbivore) and *Sorex araneus* (insectivore).

Samples from twenty individuals per species were analyzed using high-throughput sequencing. Host identification utilized mitochondrial markers (12SV5 and COI), while diet composition was assessed using ITS2 and trnL for plants, and 12S_EW (earthworm-specific) and COI for animal prey. DNA extraction protocols were optimized, and bioinformatics pipelines enabled taxonomic assignment and diversity analyses. Comparative analyses included Venn diagrams, principal component analysis, Shannon diversity index, and richness metrics. Results showed that COI enabled unambiguous species-level assignment in both fecal and stomach content samples. For *M. arvalis*, ITS2 and trnL markers revealed a diet dominated by Poaceae and Fabaceae, with no significant differences between sample types. For *S. araneus*, earthworms were part of a diet alongside arthropods and mollusks; 12S was more sensitive for earthworm detection, though COI also detected earthworms. Substantial taxonomic overlaps and no significant differences in diversity or richness metrics were observed between fecal and stomach content samples.

Fecal eDNA metabarcoding proved to be a powerful, non-invasive tool for host and diet identification, overcoming ethical and methodological limitations of traditional methods. The findings support broader adoption of fecal-based eDNA analysis in wildlife studies and regulatory risk assessment, with recommendations for marker selection and sequencing strategies. Future work will focus on increasing sequencing depth and applying more specific primers to increase species detection, especially for arthropods. Integrating molecular approaches into environmental risk assessment frameworks is a promising option that offers scalable solutions for monitoring and assessing wildlife exposure.

1.10.P-We025 Ecotox: An Outlier or Way-out?

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The world and scientists are making a push to tackle one of the fastest growing section of the earth planetary boundary - novel entities. Companies such as “A” and “B” take the lead in the production of chemical and chemical products. One notable example is the merger between “B” and “C”, accruing all assets, including the famous pesticide protection product - “D”, globally debated amid the continued use and acceptance in EU and rejection in the US, fortified with law suit amounting to millions of dollar in charges to affected individuals. Scientists such as “E” and “F” continue to lead research and scientific studies to unravel the earthly, human and environmental challenges caused by the proliferation of these chemicals. It has turned out that we are the problem of our present conditions, covered by greed, hard-work and dedication, especially involving early career scientists. Is Ecotox an outlier or a solution to the globally interconnected challenges of pollution, climate change and biodiversity loss? Time will definitely tell. Codes available upon request.

1.10.P-We026 Integrating Alternative and Refined Approaches to Bisphenol Toxicity in *Xenopus laevis*
Fábio Miguel Ribeiro Gomes, Miguel Oliveira and Isabel Lopes, University of Aveiro, Portugal

The increasing release of plasticizers into aquatic ecosystems raises concern for wildlife. Bisphenol A (BPA) is widely regulated due to its environmental persistence and bioaccumulation. Bisphenol S-methacrylate (BPS-MAE) has been proposed as a replacement, though its ecotoxicity remains poorly characterised.

Amphibians are highly susceptible to contaminants, yet the use of live organisms requires ethical consideration. Implementing the 3Rs (Replacement, Reduction and Refinement) is therefore essential to ensure ecologically relevant and ethically aligned testing approaches.

This work compares BPA and BPS-MAE toxicity in *Xenopus laevis* under a 3Rs-based framework. A6 cells were exposed for 12 and 96-h to assess (Replacement and Reduction) cytotoxicity (MTT), genotoxicity (Comet assay) and biochemical biomarkers (CAT, GST, LDH and TBARS). NF48 tadpoles were exposed for 96 h to determine LC₅₀ and EC₅₀ values, used to define sublethal ranges, followed by 12-h behavioural assays (Reduction and Refinement) using the HeMHAS and ZebraBox systems.

In vitro LC₅₀ values were 10.3 mg L⁻¹ for BPA and 27.5 mg L⁻¹ for BPS-MAE. The Comet assay showed significant DNA damage from 0.15 mg L⁻¹ BPA. In vivo, BPA was consistently more toxic than BPS-MAE (LC₅₀ = 12.1 vs. 18.1 mg L⁻¹; EC₅₀ = 9.0–10.5 vs. 17.3–20.3 mg L⁻¹). Behaviourally, BPA-exposed tadpoles aggregated in contaminated zones at 7 mg L⁻¹, while BPS-MAE induced significant avoidance from 9.3 mg L⁻¹. Reduced mobility under BPA exposure clarified this “false preference”, suggesting impaired escape capability. These results show that although BPS-MAE is less potent than BPA, it still induces toxicity across molecular, cellular, organismal and behavioural levels, and should not be regarded as a safe alternative. Integrating in vitro assays with refined behavioural endpoints demonstrates how 3Rs-oriented methodologies can deliver ethical and ecologically realistic assessments of emerging contaminants in amphibians.

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1.10.P-We027 Developmental and behavioral effects of two synthetic glucocorticoids, fluticasone propionate and clobetasol propionate, in zebrafish embryos

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Synthetic glucocorticoids have been used in human and veterinary medicine for decades to treat inflammatory pathologies including rheumatoid arthritis, asthma or Covid-19. High consumption of these pharmaceuticals combined with incomplete removal by water treatment plants result in the contamination of aquatic systems, at concentrations in the ng/L range in surface waters up to dozens of µg/L in worst cases in effluents. Despite their environmental occurrence, their effects on endocrine signaling in aquatic organisms remain understudied.

Glucocorticoids act through activation of the glucocorticoid receptor (GR), which is involved in the regulation of essential physiological processes such as stress response, immune functions, development and behavior. In the present study, we investigated the molecular, developmental and behavioral effects of two synthetic glucocorticoids, fluticasone propionate (FP) and clobetasol propionate (CP), in zebrafish embryos, using the fish embryo toxicity assay (OECD TG 236) and a dark-light transition assay (DLT) at 120 hpf.

FP and CP induced a precocious hatching of embryos (at concentrations > 1 µg/L) and developmental effect on swim bladder inflation, without affecting the survival of embryos at concentrations up to 100 µg/L. The expression of a GR-regulated gene, i.e., *fkbp5*, was also quantified in embryos. At the tested concentrations, a concentration-dependent increase of *fkbp5* gene expression was observed in zebrafish embryos exposed to FP or CP. In the DLT assay, FP and CP disturbed locomotor activity by increasing the total distance traveled during the dark phase and the time spent in movement during both dark and light phase, while decreasing the velocity of embryos. Altogether, these behavioral changes may result from the activation of the GR signaling pathway. In the present study, molecular, developmental and behavioral effects of two synthetic glucocorticoids were shown in zebrafish embryos, confirming that glucocorticoids are of concern for developing fish. Eventually, this study highlights the importance of considering the effects of chemicals, not only glucocorticoids, on the GR signaling pathway and their effects on development and behavior in fish.

Disclaimer/Disclosure:

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1.10.P-We028 Behavioral Impact of Road Dust and Tire Wear Particle on Gammarids and Potential Functional Consequences

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Tire Wear Particles (TWPs), a major component of road dust (RD), pose a significant threat to aquatic ecosystems. Abrasion of tires on roads generates microplastic-like particles that are transported to freshwater ecosystems via storm runoff. While the lethal effects of TWPs have been established for some species like Coho Salmon, their sublethal impacts on other aquatic organisms remain poorly understood. Moreover, in a global climate change context, the effect of rising temperature coupled to pollution exposure is lacking. This study investigates the behavioral alterations in gammarids (*Gammarus fossarum*), a ubiquitous sentinel species, following long-term exposure to road dust contamination for two different temperatures (12°C and 16°C). Over 21 days, we measured weekly changes in activity levels (percentage of active time), swimming speed, distance traveled, and nearest neighbor distance (NND) using video tracking. Additionally, functional effects were assessed by quantifying stress enzyme and acetylcholine esterase. Our results demonstrate a noticeable decrease in distance traveled, speed, active time, and interaction with nearby neighbors after 21 days of exposure in contaminated gammarids at 12°C. Furthermore, we observed changes in stress enzyme activity between contaminated and control at 12°C. We hypothesize that these behavioral changes may be attributed to energy reallocation towards detoxification processes, potentially compromising energy available for essential activities like swimming. This reduced swimming capacity could increase predation risk and negatively impact gammarid population dynamics. Future research will focus on assessing the vulnerability of contaminated gammarids to predation cues and exploring the behavioral effects of TWPs on other aquatic species.

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1.10.P-We029 Effects of Benzo[a]pyrene on Swimming Behaviour of Brown Trout Assessed Using Non-invasive Approaches

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Aquatic environments are increasingly impacted by chemical contaminants arising from human activities, among which benzo[a]pyrene (BaP), a widespread polycyclic aromatic hydrocarbon (HAP) generated by the complete combustion of organic materials, is of major concern. Behavioural analysis provides a sensitive method for detecting sublethal toxic effects in fish, making them valuable tools for ecological risk assessment. This study investigated the impact of BaP on the swimming behaviour of brown trout (*Salmo trutta*) with the aim of identifying locomotor alterations early warning signs of toxic stress. Juvenile brown trout received a single intraperitoneal injection of BaP (0.5 mg/kg or 10 mg/kg), and their swimming behaviour was quantitatively assessed four days post-exposure using non-invasive video tracking. Key parameters analysed included swimming speed, mean acceleration, total distance travelled, and overall activity level. This approach enabled the collection of reproducible behavioural data without inducing handling stress during observation. The results revealed a clear, dose-dependent reduction in locomotor activity following BaP exposure. Exposed trout exhibited lower swimming speeds, reduced acceleration, and shorter distances travelled. Beyond locomotor impairments, BaP exposure was associated with a suppression of anxiety-related behaviours, leading to decreased vigilance. These behavioural alterations suggest that exposed individuals may face an increased risk of predation in natural environments. Overall, this study highlights swimming behaviour as a valuable endpoint for detecting contaminant-induced functional impairments and contributes to improving ecological risk assessments in freshwater ecosystems.

1.10.P-We030 When Colours Meet Plastics: Behaviour Gets Messy

Valérie Pires, Mónica Almeida, Isabel Lopes and **Miguel Oliveira**, CESAM, University of Aveiro, Portugal

Growing demand for visually appealing food products has intensified the use of artificial food dyes (AFDs), many of which are now recognized as emerging contaminants. Concerns about their potential effects are shared by consumers, environmental scientists and food safety authorities, who increasingly emphasize the need for periodic re-assessments of their safety.

At the same time, the extensive use of plastics in food packaging and single-use applications contributes to the release of micro(nano)plastics (MNPs) into the environment, where they may interact with AFDs and potentially modulate their environmental fate, behaviour, and biological effects.

To explore these potential interactions, zebrafish (*Danio rerio*) embryos and larvae were exposed to some of the most widely consumed AFDs and model MNPs (e.g., polyethylene terephthalate, PET) at environmentally relevant concentrations, both individually and in combination, from early development up to 8 days post-fertilization. Throughout exposure, key developmental parameters (e.g., survival, hatching, malformations and heart rate) were monitored, and larval locomotor and stress-related behaviours (e.g., swimming performance) were assessed using a set of complementary behavioural assays.

Overall, both contaminants induced significant physiological and behavioural alterations even at relatively low concentrations. The co-exposure assays revealed that interactions between contaminants can modulate the effects observed in individual exposures.

These findings suggest that AFD–MNP interactions may play a key role in shaping toxicity during early fish development, highlighting the need to integrate co-exposure scenarios into future environmental risk assessments and regulatory evaluations. Ultimately, these results provide a timely contribution to understanding how emerging contaminants jointly influence aquatic organisms, with broader implications for consumer safety and ecosystem health.

1.10.P-We031 Zebrafish adaptive strategies across development: Integrating Behavioural, Physiological, and Transcriptomic Profiles

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Zebrafish were reared from early life stages to adulthood to investigate how individual coping styles emerge as adaptation strategies and how they persist across development. Of particular interest are bold and shy coping styles, because they represent fundamental behavioural strategies linked to stress responsiveness, fitness-related traits, and differential sensitivity to environmental challenges. At multiple ontogenetic stages, fish were phenotyped and sorted into bold and shy profiles using standardized behavioural criteria. Survival, growth trajectories, and sex ratios were quantified to determine whether personality type was associated with differential physiological or developmental patterns. Behavioural assessment was conducted using the light–dark test at each stage to evaluate consistency and divergence in coping styles through time. Following behavioural assays, individuals were sampled for transcriptomic analysis to link phenotype with underlying molecular signatures. RNA sequencing was performed to characterize gene expression patterns associated with coping style and developmental progression. These data provide a basis for identifying pathways potentially associated with behavioural differentiation and life-history variation. Preliminary observations suggest possible stage-specific behavioural and transcriptional distinctions between coping styles. This integrated approach advances the understanding of how behavioural phenotypes relate to developmental, physiological, and molecular traits in zebrafish.

Disclaimer/Disclosure:

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1.10.P-We032 Night Lights, Broken Rhythms: ALAN Effects on Zebrafish Larval Sleep and Sensorimotor Behaviour

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Artificial light at night (ALAN) is an emerging environmental stressor in urban aquatic ecosystems, capable of disrupting circadian regulation and altering behaviour through interference with melatonin-mediated pathways. Despite its global expansion, the behavioural consequences of ALAN, particularly in early life stages, remain understudied. In this study, zebrafish (*Danio rerio*) embryos were exposed to environmentally relevant ALAN intensities (1 and 10 lux) for seven days, and behavioural parameters associated with sleep regulation were evaluated to test whether ALAN exposure induces sleep deprivation. All behavioural assays were conducted during the daytime light phase, when zebrafish larvae are normally active, enabling the detection of potential compensatory sleep responses. Sleep-wake dynamics, including minutes of sleep per hour, and the number and length of sleep bouts, were quantified using Ethovision XT 16 software, providing a sensitive measure of circadian and neuroendocrine disruption. In parallel, the kinematics of the acoustic startle response (ASR) were analysed using the Zebra_K+ platform to evaluate changes in arousal threshold and short-term habituation, a neurobehavioural process associated with non-associative learning. Larvae exposed to 10 lux showed a significant increase in the number of sleep bouts, indicating fragmented sleep or altered arousal, whereas 1 lux increased total sleep duration despite unchanged immobility bout number. Moreover, larvae exposed to 10 lux showed a reduced ASR relative to controls with no changes in the habituation process, suggesting an increase in the arousal threshold but no alterations in cognitive function. Overall, our findings identify ALAN as a potential disruptor of neurobehavioural function during early development. These results reinforce the value of behavioural endpoints as sensitive early-warning indicators of neurotoxicity and highlight the need to integrate light pollution into ecological and regulatory assessment frameworks.

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1.10.P-We033 Small Doses, Big Effects: Low Sertraline Levels as a Behaviour Modulator in Fish

Márcio Barreto, Carla Ferreira Melo, Miguel Oliveira and Isabel Lopes, University of Aveiro, Portugal

Antidepressants are widely prescribed pharmaceuticals, increasingly recognized as contaminants of emerging concern. These drugs enter aquatic systems through patient excretion and improper disposal, combined with limited efficiency of wastewater treatment plants in their removal. Designed to modulate conserved serotonergic pathways, SSRIs can act on molecular targets and potentially affect non-target aquatic organisms. In fish, serotonergic signalling regulates essential behaviours, such as social interaction, stress responses, and predator avoidance, which can be disrupted by neuroactive substances present in the environment. Sertraline (SER), a widely used SSRI, has been detected in surface waters worldwide, at concentrations ranging from low to several hundred ng L⁻¹, where it may induce physiological and behavioural alterations in animals. This study aimed to evaluate the effects of environmentally relevant concentrations of SER on behavioural and physiological responses of early-life stages of zebrafish (*Danio rerio*). Embryos were exposed from 4 hours post-fertilisation (hpf) up to 8 days post-fertilisation (dpf) to six SER hydrochloride concentrations (0.16–500 µg L⁻¹) prepared in a fivefold dilution series, plus a negative control (E3 medium only). Spontaneous tail coil movements were quantified as an early neurotoxicity biomarker (at 24 hpf), heart rate was assessed as a physiological endpoint (at 48 hpf), and swimming profiles under dark and light conditions (at 8 dpf) were assessed as behavioural endpoints. Overall, SER exposure increased heart rate at all tested concentrations, indicating potential effects on the cardiovascular system. Under dark conditions, a decrease in locomotor activity (total swimming distance and average speed) was observed at 100 and 500 µg L⁻¹ SER. Thigmotaxis-related endpoints (distance and time spent in the outer zone of the test arena), as well as the time spent inactive decreased at 500 µg L⁻¹. Larvae's stress response was also altered after exposure to 20, 100, and 500 µg L⁻¹ SER which induced reduced total distance swam and average speed, and increased inactivity during light-to-dark transition tests. Organisms exposed to 20 µg L⁻¹ showed decreases in time and distance spent in the outer zone of the arena during stress tests. These results highlight the potential of SER to induce behavioural alterations even at low concentrations, and the potential to alter stress-related behavioural traits during early development.

1.10.P-We034 Exploring In Vitro Testing for Comparative Cellular Toxicology: Challenges, Opportunities and Future Directions

Isabelle Kavanagh, University of Birmingham, United Kingdom

Inter- and intra-species variation in chemical sensitivity presents a key challenge for chemical safety assessment for both humans and the environment. Regulatory toxicology has traditionally relied on a limited set of model organisms, providing only a narrow representation of the susceptibility of species intended to reflect the vast biodiversity of our ecosystems. Evaluating the current use of in vitro systems to characterise cross-species cellular responses is therefore critical, as these approaches allow exploration of the cellular and mechanistic drivers of variation. Such insights can generate predictive information to support more protective and broadly applicable chemical safety assessments while reducing reliance on animal testing.

The first phase of this PhD project focuses on reviewing the literature of the current applications of in vitro testing in regulatory toxicology. This includes evaluating the choice and characteristics of commonly used cell lines, assessing their relevance across species, and highlighting methodological strengths, limitations, and opportunities for cross-species comparison. The review also examines the translation of laboratory-based findings into regulatory adoption, including successful examples where in vitro approaches have reduced reliance on whole-animal models, and identifies future directions for advancing in vitro methods for predictive toxicology.

This work establishes a foundation for subsequent comparative cross-species cellular toxicology, with standardised experimental designs across multiple species enabling quantification of cellular responses under consistent conditions. It will also provide a basis for integrating genetic and evolutionary perspectives, exploring how mechanistic variation aligns with species relatedness and informing more predictive, phylogenetically informed approaches to chemical safety assessment.

1.10.P-We035 Molluscan Nuclear Receptors and Their Ligands: What We Know, What We Don't, and What Comes Next

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Nuclear receptors (NRs) regulate development, metabolism, and reproduction, yet current endocrine-disruptor assessment remains centered on vertebrate EATS pathways. Our literature review shows that mollusks retain some vertebrate-like NR complements (RXR, RAR, THR, PPAR), unlike many invertebrates, which have lost key receptors, and that RXR is the only well-characterized molluscan NR known to be directly activated by contaminants such as organotins. In contrast, RAR, THR, and PPAR show altered ligand binding, limited transcriptional characterization, and unresolved physiological roles, representing a major knowledge gap. Based on the results of our literature review, our next step will be to identify life-stage-specific NR responsiveness in *Lymnaea stagnalis* using endogenous and xenobiotic ligands, then investigate RXR crosstalk with partner receptors, and evaluate cross-phylo conservation using zebrafish. This research will deliver a mechanistic mapping of NR signaling in mollusks, clarify how endocrine disruptors act across taxa, and strengthen the biological relevance of future ecotoxicological assessment frameworks.

Disclaimer/Disclosure:

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1.10.P-We036 Emerging mycotoxins and 3D HepaRG liver models: comparative mechanistic insights from mono- and tri-cultures in the context of New Approach Methodologies

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Mycotoxins, secondary metabolites produced by various species of fungi, pose significant health risks to humans and animals. These toxins, which commonly contaminate foods such as cereals and fruits, are stable and resistant to degradation during food processing. Over the past few decades, thousands of mycotoxins have been identified and characterised. Only a limited number of these toxins are regulated, and emerging toxins remain unregulated due to insufficient toxicological data.

The MYCO3D project, funded by the Brittany Region, emphasises the application of New Approach

Methodologies (NAMs) to assess the hepatotoxic potential of emerging mycotoxins, including Enniatins A and A1, as well as Alternaria mycotoxins Alternuene and Tentoxin. Using HepaRG spheroids, our results show that Enniatins A and A1 induced significant toxicity, oxidative stress, disruption of mitochondrial transmembrane potential, and steatosis following 48 hours of treatment. Whereas a 3-day treatment with Alternaria mycotoxins at concentrations up to 100 µM did not induce any cytotoxic effect. However, a 10-day chronic exposure to Alternuene and Tentoxin, with media renewal every 48 hours, resulted in significant decreases in cell viability only in cells treated with Tentoxin.

To further examine the cytotoxic effects of mycotoxins, we utilised more sophisticated liver organoid models that include HepaRG cells, primary human macrophages, and LX-2 stellate cells. This advanced liver organoid system enables the evaluation of mycotoxins' effects on complex inflammatory and fibrotic processes. Unlike the monoculture model, organoids composed of HepaRG, macrophages, and LX-2 cells showed greater resistance to Enniatins, with cell viability staying above 40% even at the highest concentration tested (10 µM). To gain deeper mechanistic insights, a transcriptomic study has been carried out using the TempO-Seq™ technology from BioClavis (UK) to investigate the molecular pathways involved in mycotoxin cytotoxicity and adaptation responses. Results are currently being processed to identify differentially expressed genes, and the impacted pathways. An overview of the results will be presented, either as a poster or an oral presentation. Overall, our results show that, unlike Enniatin A and A1, Alternaria mycotoxins Alternuene and Tentoxin do not cause adverse effects. This statement will be further investigated upon transcriptomics data treatment.

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1.10.P-We037 Assessing Toxicometabolomic Responses of Daphnia Magna to Pharmaceutical Mixtures in Municipal Wastewater Treatment Plants from Costa Brava (Catalonia, Spain)

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The increasing scarcity of freshwater highlights the urgent need to identify alternative sources. Wastewater represents a promising option but requires advanced treatments to remove wastewater-borne contaminants, particularly contaminants of emerging concern. Previous targeted chemical studies have shown that, although tertiary treatments enhance removal efficiency, they do not ensure the complete elimination of pharmaceutical residues. Based on this evidence, the present study aims to address the hypothesis that residual concentrations persisting after treatment are sufficient to trigger measurable biological effects in exposed organisms.

To evaluate this hypothesis, a comprehensive sampling campaign was performed to assess the efficiency of 14 wastewater treatment plants located along the Costa Brava (Spain) to remove pharmaceutical contaminants. At each facility, effluent samples were collected at two stages (secondary and tertiary treatment) to capture differences in removal performance. A multiscale approach was applied integrating the concentrations from chemical analyses with a set of behavioural responses in *Daphnia magna* including startle, habituation, basal locomotor activity, visual motor response and phototaxis. In parallel, metabolomic data was obtained from the exposed organisms via ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) to characterise changes in key biochemical pathways.

Finally, data analyses were conducted using multivariate statistics (PCA, PLS-DA) followed by pathway enrichment studies to identify the affected metabolic processes. The obtained results revealed clear clustering patterns that distinguished wastewater effluents according to both treatment characteristics and local population pressures. Sites serving larger populations exhibited higher pharmaceutical loads and correspondingly stronger biological responses, while differences in treatment processes significantly influenced the magnitude and nature of observed effects.

Overall, this integrated approach provides novel insights into the ecotoxicological risks associated with wastewater reuse highlighting the urgent need for more advanced tertiary treatments and the importance of stricter regulations to minimize pharmaceutical contamination, particularly when effluents are reused for human-related applications. Even at trace levels, pharmaceuticals may compromise both ecosystem integrity and human health.

1.10.P-We038 Early Life Stress Rewrites the Script of Coping

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Early-life stress (ELS) can shape animal behaviour and physiology, yet its influence on the development of coping styles remains poorly understood. In this context, the presence of the antidepressant paroxetine (PAR) in aquatic environments is concerning, as it acts on conserved serotonergic pathways that play a key role in behavioural regulation.

This study aimed to determine how ELS, caused by abiotic (AB) stressors or environmental levels PAR, affects later-life coping styles (bold vs. shy) in zebrafish. To address this question, zebrafish larvae were exposed to sequential abiotic (AB) stressors at key developmental stages (4-, 7- and 15-days post fertilization - hpf), while embryos were exposed to PAR during embryogenesis (up to 48 hpf). After stress exposure, AB and PAR fish were reared under optimal conditions until the juvenile stage (45 dpf). At this stage behavioural profiling, neurochemical analysis, gene expression, and cortisol measurements were used to assess long-term consequences of ELS. Results show that bold and shy fish differ intrinsically in activity levels and monoaminergic regulation, and that ELS modifies these coping styles without altering their overall proportion. The 15 dpf stage was the most sensitive to AB stress, with shy juveniles exhibiting more behavioural, neurochemical and endocrine alterations than bold counterparts. PAR effects were dose-dependent, with shy phenotypes more affected by high PAR and bold phenotypes by low PAR concentrations.

Overall, ELS generated distinct stress-modified sub-phenotypes within bold and shy categories, with shy fish higher susceptibility to long-term programming. These findings highlight phenotype-specific developmental plasticity and improve understanding of how early adversity and neurochemical pollutants shape behavioural coping strategies. More research is needed to explore the adaptive potential of these ELS-modified coping styles in response to environmental contaminants, particularly for shy individuals.

1.10.P-We039 Behavioral Alterations of the Amphipod Gammarus pulex During Toxicokinetic Processes: The Role of Receptor Binding

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Insecticides can not only target specific pest species but also have an impact on non-target organisms, such as aquatic invertebrates. In regulatory environmental risk assessments, toxicokinetic-toxicodynamic (TKTD) models are accepted for predicting time-dependent biological effects. Toxicokinetic studies are crucial for understanding chemical uptake and elimination dynamics, whereas the impact of these chemicals (toxicodynamic) is mainly assessed using lethal endpoints (i.e., immobility and mortality). However, behavioral endpoints can often be observed at concentrations below the lethal threshold and can indicate sublethal stress.

To explore toxicodynamics and sublethal effects, we investigated the relationship between internal concentrations and behavioral alterations using two polar neuroactive insecticides: the neonicotinoid thiacloprid and the butenolide flupyradifurone, both targeting the nicotinic acetylcholine receptors (nAChRs). Toxicokinetic analysis (online-SPE LC-HRMS/MS) revealed an elimination resistant fraction

for thiacloprid suspected to irreversibly bind to the nAChR, while flupyradifurone was fully eliminated.

Behavioral responses, quantified as total distance moved, were continuously monitored during both the uptake and elimination phases over 7 days to identify toxicokinetic-related changes in movement patterns. Notably, thiacloprid exposure resulted in a decrease in locomotion during the uptake phase, as well as a suppression of the circadian activity rhythm with incomplete recovery relative to controls during the elimination phase, consistent with irreversible receptor binding. Additionally, short-term behavioral assays were conducted at the end of each phase under both environmentally relevant and effect-inducing concentrations, assessing concentration-dependent responses to alternating light and dark conditions.

Our findings demonstrate the practical applicability of behavioral ecotoxicological approaches to toxicokinetics. With this study, we aim to improve the mechanistic understanding of chemical-induced behavioral alterations during uptake and elimination, linking toxicokinetics with sublethal effects.

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1.10.P-We040 The Rise of New Approach Methodologies (NAMs) for Environmental Safety Assessment of Chemicals: Current Status, Challenges, and Strategies for Broader Adoption of NAMs

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Traditional ecotoxicity testing relies heavily on vertebrate animal models, which are resource-intensive, low-throughput, and raise ethical concerns under the 3Rs principle (Reduction, Refinement, Replacement). New Approach Methodologies (NAMs) including in silico, in chemico, in vitro and ex vivo offer promising alternatives for environmental hazard assessment. While NAMs have gained traction in human health risk assessment, their regulatory acceptance for environmental safety remains limited due to technical, scientific, and policy barriers. This proposed platform presentation will present an overview of a literature review. The review's objectives were to: (1) systematically review the current state of NAMs for aquatic toxicity assessment, (2) identify gaps in NAM readiness and endpoint coverage, and (3) provide recommendations to accelerate regulatory acceptance and integration of NAMs into environmental safety frameworks.

A systematic literature review was conducted using PubMed followed by the SWIFT-Active Screener tool, focusing on NAMs applicable to aquatic organisms (fish, amphibians, marine mammals). The review excluded terrestrial and invertebrate models. Search terms considered within the scope of the review included in silico, in chemico, in vitro and ex vivo. While embryo assays are not considered a NAM by all geographical regions and stakeholders, information from existing embryo assays was also reviewed to reduce the reliance on protected vertebrate life stages.

The screening identified 4,275 unique articles; 1,310 included for full-text review. A database containing all the reviewed information is freely available and contains 18 metadata fields including for example the captured assay type, species, endpoints, and readiness level.

This review provides a comprehensive roadmap for transitioning toward animal-free environmental safety assessment. By addressing gaps in readiness, standardization, and mechanistic integration, NAMs can become central to next-generation risk assessment, supporting global initiatives to phase out vertebrate testing.

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1.10.P-We041 Are Behavioural Ecotoxicity Endpoints Relevant at the Population Level? Evidence-Based Insights for Environmental Protection

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A substantial body of evidence exists demonstrating that exposure to environmental contaminants can alter animal behaviour. Moreover, methodological and technological advancements, as well as increasing standardisation, mean that behavioural ecotoxicity studies are more rigorous and reliable than ever before. Despite this, behavioural data are still seldom used in the risk assessment and regulation of chemicals. This is partly due to a lack of clarity among some stakeholders about whether changes in behaviour at the individual level result in population-level outcomes. To address this, we first consider the state of evidence within the field of behavioural ecotoxicology linking individual-level behavioural alterations with population-level consequences. We then assess the evidence from behavioural ecology and other neighbouring fields that supports this link. Further, we evaluate whether some behavioural endpoints are more easily tied to population-level changes than others. In this regard, we propose combining insights from two complementary ecological frameworks—the functional trait framework and the limiting traits framework—to evaluate which behaviours should be prioritised in ecotoxicological research and regulatory efforts. We contend that the link between behavioural changes and population-level outcomes is evident, with behavioural endpoints representing a highly valuable yet so-far underutilised line of evidence in applied environmental protection.

1.11.A From Molecules to Models: Integrating Mechanistic Insights and Quantitative Modelling into Predictive Ecotoxicology

1.11.A.T-01 In Vitro Hepatic Microsomal Michaelis-Menten Parameters In Biotransformation: Do They Scale Allometrically?

Luke Ockhuijsen¹, Ad M. J. Ragas² and Jan A.J. Hendriks¹, (1)Radboud University, Netherlands, (2)Environmental Science, Radboud University, Netherlands

Extrapolating xenobiotic biotransformation parameters between species is challenging due to species differences. At the organism level, pharmacokinetic rate constants are believed to scale allometrically to body weight with a power of $\frac{3}{4}$, akin to metabolic rate constants. However, the underlying contributing factors for biotransformation scaling are unclear. Altogether, this understanding builds up to predicting xenobiotic biotransformation through a quantitative in vitro to in vivo extrapolation (QIVIVE) approach, combining allometric scaling relationships related to liver physiology and in vitro xenobiotic biotransformation. This study explores the previously unexplored potential of allometric scaling for hepatic microsomal Michaelis-Menten parameters. Hepatic microsomal Michaelis-Menten parameters, viz., affinity (K_m), maximum velocity (V_{max}), and in vitro clearance (CL_{int}), were examined for the xenobiotic biotransformation enzymes Cytochrome P450 (CYP) and UDP-glucuronosyl transferase (UGT). To ensure validity, data for 74 mammals per substrate biotransformation pathway were collected through a literature search and available databases. Ordinary linear regressions per pathway for each parameter showed that only 10% of the regressions scaled statistically significantly to body weight. Averaging all scaling exponents of significant regressions revealed that hepatic microsomal Michaelis-Menten parameters are independent of body weight. The average scaling exponent for each Michaelis-Menten parameter did not deviate from 0. Therefore, differences in other factors like enzyme abundance or liver physiology between species might be contributors to the observed $\frac{3}{4}$ exponent for allometric scaling relationships of in vivo xenobiotic clearance. Characterisation of underlying mechanisms at an enzyme-substrate level might ultimately improve understanding and estimation of xenobiotic clearance for mammals in QIVIVE approaches.

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1.11.A.T-02 MIE, Myself and IC50: The Importance of the Molecular Initiating Event for the Difference in Carbaryl Sensitivity of Aquatic Arthropods

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Species differ in their sensitivity to pesticides, but the mechanisms behind these differences are only partly understood. The sensitivity of an organism for a compound is the product of both toxicokinetics, what the organism does to the compound, and toxicodynamics, what the compound does to the organism. Although variation in toxicokinetic processes in aquatic arthropods has been linked to the interspecies differences in sensitivity to pesticides, the role and variation in toxicodynamic processes is less well characterised. Addressing this knowledge gap, we examine a key toxicodynamic component: the molecular initiating event (MIE). We characterize the difference in interaction at the MIE of the carbamate carbaryl in six aquatic arthropod species to better understand its role in driving the difference in sensitivity. The MIE of carbaryl is the binding and inhibition of acetylcholinesterase (AChE). Structural variation in AChE across species may influence this interaction. To quantify the interaction at the MIE, homogenised samples of six aquatic arthropod species were exposed to a range of carbaryl concentrations and AChE activity was measured using the Ellman method adapted for plate reader. We derived the half-maximal inhibitory concentration (IC50), the bimolecular rate constant and the decarbamylation rate. The sensitivity of the organisms was reflected by the effect threshold in a reduced General Unified Threshold Model of Survival (GUTS). For the tested species, the Effect Threshold showed a strong positive correlation with the IC50.

This indicates that the interaction between carbaryl and AChE at the MIE is an important driver of sensitivity. Additional tests will show whether (de)carbamylation rates will correlate even stronger with the sensitivity of the organisms. These results also show that in vitro (AChE inhibition) assays could potentially be used to predict species sensitivity to carbamates.

1.11.A.T-03 What's Really Stopping the Head-AChE? Implementing Enzyme Inhibition in TKTD Model for Carbaryl

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Linking the adverse outcome pathways (AOP) framework with effect model approaches will enhance our ability to robustly extrapolate across different levels of biological organization. Due to their increasing acceptance in Environmental Risk Assessment (ERA) and their continuously growing application in the field, mechanistic effect models like toxicokinetic-toxicodynamic (TKTD) models, seem the most logical choice for this endeavor. Therefore, we implement a TKTD model for carbaryl and *Gammarus pulex*. Informing the model with in vivo AChE activity measurements enables us to explicitly model the toxicodynamic process that is assumed to be driving toxicity (i.e. AChE inhibition).

Our results confirm the inhibiting effect of carbaryl on the target molecule AChE in *G. pulex* and display its recovery dynamics which together drive the effect on the organism's survival. In the next step, we use the TKTD model to combine these quantitative measurements on the molecular level with data on internal concentrations and the survival of *G. pulex* exposed to carbaryl, enabling us to create a quantitative link across multiple levels of biological organization.

As the development of our TKTD model is informed by data from different levels and scales (i.e., toxicokinetics, enzyme activity, and individual survival), we provide a robust tool to assess the toxicity of carbaryl and other AChE inhibitors with high predictive power. In doing so, we provide a versatile contribution to advance towards predicting adverse effects in non-target species, based on molecular endpoints, thereby supporting the AOP framework.

Disclaimer/Disclosure:

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1.11.A.T-04 Expanding the Chemical Domain of Applicability of a Cross Species Quantitative Adverse Outcome Pathway for Activation of the Aryl Hydrocarbon Receptor Leading to Early Life Stage Mortality

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental chemicals that induce toxicity by activating the aryl hydrocarbon receptor (AhR). Species differ dramatically in sensitivity to AhR activation by dioxin-like chemicals (DLCs) and PAHs. A predictive model linking AhR activation by DLCs to early life-stage mortality in birds and fish has been developed to aid in risk assessment, however this model is not applicable to PAHs. This research aims to 1) expand the domain of applicability of the existing model to PAHs and 2) use in silico approaches to explore molecular mechanisms underlying differences in species sensitivity to potentially incorporate in silico predictions into the model. A standardized in vitro reporter gene assay was used to develop species sensitivity distributions for transactivation of the AhR across species. To determine whether in vitro differences in sensitivity to AhR activation correspond with differences in sensitivity to early life-stage mortality, embryos of a subset of fish and avian species were exposed to PAHs by microinjecting into the yolk sac of embryos. Lastly, in silico analysis was performed to determine binding potential energies and investigate the role of receptor subdomains in differences in species sensitivity. Species differences 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 in fish (LD50; $p = 0.007$, $R^2 = 0.86$) and

birds ($p = 0.007$, $R^2 = 0.91$). Adjustment factors were calculated for PAHs in birds and fish to enable predictions of PAH toxicity to be made using the existing predictive model. Predicted LD50s were within 3.9-fold of measured LD50s across birds and fish, on average. There is a significant correlation between the EC50 for activation of the zebrafish AhR2 by 22 structurally diverse ligands and *in silico* binding affinity ($p = 0.0002$, $R^2 = 0.57$) which suggests that this predictive model can be further expanded to generate predictions using *in silico* methods, however cross-species validations are ongoing. Due to the abundance of PAHs in the environment 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.

1.11.A.T-05 When are Repeated Pesticide Exposures Independent? A Species-Specific Approach Using GUTS-based Depuration Times

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Pesticide persistence inside organisms is far less understood than persistence in soil or water, yet internal elimination and recovery processes critically shape how organisms respond to repeated exposure pulses in agricultural landscapes. When elimination is slow, sequential applications can lead to carry-over toxicity, but current risk assessment frameworks focus mainly on external exposure declines and overlook internal toxicokinetics. This study therefore evaluates how recovery dynamics, quantified through toxicokinetic–toxicodynamic (TKTD) parameters, influence the minimum safe interval between applications of products containing the same active ingredient.

We conducted a targeted literature review of published TKTD parameters for pesticides, following PRISMA principles. Forty-one studies met all criteria, from which we extracted toxicokinetic rate constants across five TKTD model types and calculated the 95% depuration time (DRT_{95}) as a cross-model indicator of recovery. Data covered 36 chemicals and 75 species spanning 37 taxonomic orders.

DRT_{95} values varied widely across species and chemical classes. Cladocerans and some aquatic insects showed the longest recovery times, whereas fish typically exhibited faster elimination. Variation within taxa was often driven by chemical class: for example, amphipods showed slow recovery from neonicotinoids but rapid depuration of pyrethroids. These patterns highlight substantial differences in vulnerability to repeated pulses, even among closely related species.

Our findings demonstrate that DRT_{95} is a mechanistically grounded and broadly applicable metric for defining minimum intervals between applications of products sharing the same active ingredient. Active ingredients with long DRT_{95} values should be limited to single annual applications, and the combined use of multiple products containing the same active ingredient should be avoided to prevent internal carry-over. Compounds with short DRT_{95} values may allow multiple applications, but stacking within a single treatment remains inadvisable. By integrating internal toxicokinetics into guidance on application timing, pesticide regulation can better reflect the dynamics of real-world exposure and reduce unintended risks for non-target organisms. Future work should quantify whether mixtures of active ingredients require additional safety margins.

1.11.B From Molecules to Models: Integrating Mechanistic Insights and Quantitative Modelling into Predictive Ecotoxicology

1.11.B.T-01 Linking Biomarkers to Life-History Traits through Response–Response Approaches in Three-Spined Stickleback (*Gasterosteus aculeatus*) Exposed to Bisphenol S (BPS)

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Freshwater ecosystems are increasingly threatened by complex mixtures of anthropogenic contaminants. Since monitoring all chemicals is unfeasible, integrative approaches are needed to evaluate mixture toxicity. Biomarkers provide a sensitive and mechanistic framework to link sub-individual alterations with higher biological responses. This study investigated the effects of bisphenol S (BPS), an emerging endocrine disruptor, on the three-spined stickleback (*Gasterosteus aculeatus*), focusing on response–response relationships between biomarkers and life-history traits.

Males and females were individually exposed for 40 days to 0, 1, 10, and 100 $\mu\text{g/L}$ BPS under controlled laboratory conditions. After 40 days, males were placed in isolated compartments with nesting materials and visual access to females for a 7-day nest-building test. Morphometric indices (HSI, GSI, NSI), biochemical biomarkers (hepatic lipid content, VTG, SPG, sperm mortality), and reproductive behaviour (nest-building) were assessed. Data were analysed using ANOVA or Kruskal Wallis tests, response–response regression

modelling, and Linear Discriminant Analysis (LDA).

In females, a significant increase in HSI at 100 µg/L BPS indicated an obesogenic response, while trends in GSI and oocyte counts suggested estrogenic activity. A four-parameter log-logistic model accurately described the relationship between GSI and mature oocytes, revealing reproductive dysfunction potentially linked to egg retention or abnormal yolk accumulation. In males, GSI decreased significantly at 100 µg/L BPS, and nest-building was delayed among individuals displaying extreme physiological profiles. The LDA score, integrating HSI, NSI, and GSI, efficiently identified these outliers and predicted nest-building performance.

Overall, this study demonstrates the relevance of response–response approaches to link physiological and behavioural responses under chemical stress. By integrating multivariate biomarker data, these methods enhance the predictive power of ecotoxicological assessments and support the development of mechanistic biomonitoring tools to improve ecological risk assessment of emerging contaminants such as BPS.

1.11.B.T-02 Temperature Effects on Species Sensitivity Distributions Across Aquatic Taxa

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Human activities introduce a wide range of chemicals into aquatic environments, where they can affect aquatic organisms and disrupt ecosystem functioning. Ecological risk assessment frameworks aim to determine concentrations at which chemicals pose no or only minimal risk to the environment, often using the Species Sensitivity Distribution (SSD) approach. However, SSDs are generally based on standardised toxicity tests conducted at specific temperatures, even though aquatic species experience natural temperature variation. This study explores how SSDs vary with respect to the temperatures at which toxicity is assessed. Acute toxicity data for fish, crustaceans, and algae were obtained from the ADORE database. 22 chemicals with sufficient data were selected for analysis. The linear Arrhenius equation was used to assess the relationship between temperature and LC50 values for species chemical pairs. To investigate temperature effects on SSDs, LC50 values were grouped by test temperature, and separate SSDs and corresponding HC50 values were constructed for each chemical where data allowed. The Arrhenius equation was also applied to describe correlations between temperature and HC50.

Temperature did not show consistent significant effects on LC50 across all species chemical pairs. However, in most cases where statistically significant relationships were found, toxicity increased with rising temperature. SSDs constructed for individual chemicals were generally similar in shape but shifted to the left with increasing temperature, indicating higher sensitivity under warmer conditions. This pattern occurred only when the same species contributed to SSDs across all temperatures. When HC50 trends could be evaluated, their temperature responses were similar to those observed for LC50.

These results show that temperature can influence both species-level toxicity and community-level sensitivity estimates. Incorporating temperature as a modifying factor in SSD-based risk assessments could therefore improve their relevance under changing environmental conditions.

1.11.B.T-03 Mechanistic Modelling of Insecticide Effects on Lepidoptera using DEB-TKTD

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Current insecticide risk assessments for endangered Lepidoptera under U.S. regulatory frameworks (US EPA) rely on safety factors often derived from honey bee data, potentially leading to inaccurate risk characterization. To address this limitation, we are developing mechanistic effect models combining Dynamic Energy Budget (DEB) theory with toxicokinetic–toxicodynamic (TKTD) approaches. The DEB framework models animal life cycles in terms of energy allocations for growth, development, and reproduction, while DEB-TKTD models additionally incorporate chemical uptake, metabolism, and lethal and sublethal effects on DEB processes. In the first project phase, the painted lady *Vanessa cardui* was used as a model species. A recently proposed DEB variant for terrestrial holometabolous insects was refined to match the growth pattern of *V. cardui*. Insecticide effects on survival and growth of three larval instars under dietary exposure were analysed using the DEB-TKTD approach. Traditionally, (DEB-)TKTD models have mainly focused on aquatic species, where exposure can be described directly by the external water concentration. For terrestrial organisms, however, uptake kinetics require explicit reformulation. Here, we build upon the recently proposed BufferGUTS model and evaluate its applicability for integrating lethal and sublethal endpoints with diet-based terrestrial exposure metrics in DEB-TKTD.

The newly generated laboratory data show clear dose-response relationships for survival and growth, with a life stage-dependent sensitivity to the insecticide. Size-dependent toxicokinetics alone could not fully explain this pattern, so additional assumptions are being explored. Preliminary modelling suggests that assuming an independent effect-slope parameter for each life stage improves fits while keeping model complexity low. Plausible physiological modes of action (pMoA) are currently being evaluated to link damage to observed growth effects. We see the integration of sublethal and lethal endpoints as a particular strength of DEB-TKTD, and ongoing experiments will help refine the model structure and clarify sensitivity patterns. Overall, this work demonstrates both the feasibility and the added value of DEB-TKTD modelling for Lepidoptera. As additional endpoints and mechanisms are incorporated, the model is expected to provide a robust basis for future risk assessments.

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1.11.B.T-04 Mechanistic Modelling of Aquatic Mesocosm Communities: Exploring Model Structures for Ecotoxicological Studies

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Mesocosms are controlled outdoor aquatic model ecosystems that enable the study of ecological responses to toxicants under environmentally realistic conditions. They capture both natural variability and the direct and indirect effects of chemical exposure at the community and ecosystem levels, making them valuable tools in higher-tier ecological risk assessment. However, the inherent complexity of mesocosm systems complicates the interpretation of observed community dynamics. Interacting biological processes, measurement uncertainty, and natural variability are confounded in the data, making it difficult to disentangle underlying mechanisms and to accurately understand the processes occurring within the mesocosms.

Mechanistic modelling provides a powerful tool, enabling better understanding of community dynamics and complex biological interactions. However, the level of detail required for such models to remain both interpretable and useful is still unclear. This research therefore aims to develop and compare versions of a mechanistic model that differ in the resolution of the food web and represent key ecological processes and food web interactions in control mesocosms without chemical treatment. Bayesian inference is applied to quantify uncertainty and enhance the interpretation of variability observed in mesocosm dynamics.

By systematically linking model complexity with predictive performance and uncertainty, this study aims to establish a framework for interpreting mesocosm observations with a quantitative approach. This work provides a foundation for future applications to toxicant exposure scenarios, ultimately improving environmental risk assessment by connecting experimental data with ecological understanding in mesocosms.

1.11.B.T-05 Quantifying the Effect of Biodiversity Loss to Freshwater Ecosystems by Linking Mean Species Abundance to Ecosystem Services

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Chemical pollution is among the five highest-ranked environmental drivers of biodiversity loss, with freshwater ecosystems among the most vulnerable. The emerging consequences of this loss remain insufficiently quantified due to the complexity of ecosystem responses. This work addresses this challenge by determining the loss of ecosystem services resulting from chemical stress-induced biodiversity loss.

As representatives of an ecosystem, we used data from three freshwater mesocosm experiments. The mesocosms were exposed to the fungicide carbendazim, the herbicide linuron, and the insecticide mixture of chlorpyrifos and lindane. To determine ecosystem services, ecosystem productivity was assessed through decomposition rates, photosynthetic activity, and macrophyte biomass production. These indicators were chosen to represent provisioning, regulating, and supporting ecosystem services. The mean species abundance (MSA) served as a metric of biodiversity. It was used to calculate the mean species abundance relationship (MSAR), which represents the loss of biodiversity as a function of chemical concentration. Functional models were then

applied to translate biodiversity decline into changes in ecosystem productivity.

It was shown that each chemical's mode of action resulted in distinct ecological responses and that ecosystem productivity decreases log-linearly with biodiversity reduction. The resulting loss of ecosystem services at the current hazardous concentration threshold for 5% of species (HC5) will be critically discussed to evaluate its safety for risk assessment. Furthermore, the implications of this loss at HC5 will be converted into monetary values to provide additional information to stakeholders. This approach enhances the integration of biodiversity-function relationships into environmental management and supports a more thorough evaluation of the impacts of chemicals on the environment.

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1.11.P From Molecules to Models: Integrating Mechanistic Insights and Quantitative Modelling into Predictive Ecotoxicology

1.11.P-We042 Identification of Ecotoxicological Endpoints for Developing Predictive QSAR Models of Naphthenic Acids in Brazilian Produced Water

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Produced water (PW) is the main effluent generated during oil and gas exploration; some of its constituents can cause damage to the aquatic environment, including naphthenic acids, phenols, BTEX, and PAH. In Brazil, according to the Brazilian National Oil Agency, 4.344 m bbl/d of oil equivalent were obtained in 2024; that production can generate between 17.38 m and 43.44 m bbl PW/d, which potentially may have a significant impact on the aquatic environment. In this context, quantifying the damage caused by PW compounds through ecotoxicology assays is crucial for determining effective strategies to treat this effluent. However, in recent years, the increasing concern about the impact and damage that these assays can cause to organisms has sparked a debate about reducing tests involving organisms, and it has encouraged the development of alternative methods for evaluating the effects that substances may have on the environment. QSAR models are crucial for determining ecotoxicology results without the need for additional assays using organisms. In this work, a strategy was developed to extract and filter data from databases to determine possible organisms and ecotoxicology endpoints with parallel curation on data to build a model to predict results of ecotoxicology tests to naphthenic acids present in Brazilian PW of the oil and gas industry. In the first step, many substances (658 compounds) were gathered with the QSAR Toolbox that were similar to the naphthenic acids chosen to be predicted. In the second step, data on organisms with more results for the substances collected in the first step were extracted, and then the two organisms with the most results were selected. In the third step, the ecotoxicology endpoints with more results were counted and ranked, and every not-conclusive result ($LC_{50} > 100$ mg/L) was removed. These steps demonstrated that *Daphnia magna* was the aquatic organism with the most compounds exhibiting ecotoxicological results for the model: EC5048h for mobility (76 compounds) and LC50 48h for mortality (49 compounds). *Raphidocelis subcapitata* was the second aquatic organism with more results: EC50 72h for growth rate (68 compounds), NOEC 72h for growth rate (64 compounds), and EC10 72h for growth rate (45 compounds). These five endpoints may provide applicable QSAR models to the naphthenic acids of PW. The following steps will be the collection of descriptor data, the development of models and the statistical validation of the results.

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1.11.P-We043 Exploring Mechanisms of Species Sensitivity to AhR Activation using In Silico Approaches
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The aryl hydrocarbon receptor (AhR) transcription factor that is activated by a wide range of structurally diverse endogenous or exogenous ligands. Species differ dramatically in sensitivity to AhR activation by various ligands including dioxin-like chemicals (DLCs) and polycyclic aromatic hydrocarbons (PAHs). Sensitivity to AhR activation by DLCs in birds is determined by two key amino acids in the ligand binding domain, however due to the AhR of fishes being less conserved the mechanisms underlying species sensitivity in fishes are not well understood. This research utilized in silico molecular docking to characterize ligand-AhR interactions and binding affinity. To determine whether differences in in silico binding affinity correspond with differences in chemical potency, molecular docking with the AhR2 of zebrafish (*Danio rerio*) was performed for 22 ligands, including 7 DLCs, 1 heterocycle, 1 benzotriazol ultraviolet stabilizer, and 4 unsubstituted, 3 alkyl-substituted, and 6 halo-substituted PAHs with available in vitro data, which yielded a significant correlation between the in vitro EC50 for AhR2 activation and in silico binding affinity ($p = 0.0002$, $R^2 = 0.57$). To investigate whether differences in binding affinity correlate with species differences in sensitivity to AhR activation, molecular docking was performed with the AhRs of 9 phylogenetically diverse fish species. While there was no significant correlation between the EC50 for DLCs and binding affinity across species ($p = 0.11$, $R^2 = 0.36$) there was a significant correlation between EC50s for AhR activation and the size of the glutamine- and acidic-rich subdomains of the transactivation domain of the AhR ($p = 0.0005$, $R^2 = 0.65$). This suggests that both the ligand binding and transactivation domains of the AhR are involved in species differences in sensitivity to AhR2 activation by DLCs. Research is ongoing to generate data in additional species and with PAHs to investigate whether the same relationship is true for PAHs. Additionally, work is ongoing to characterize differences in binding position and specific amino acid interactions across species and ligands, and the role of the ligand binding and transactivation domains in driving differences in species sensitivity. This understanding could potentially be used to further improve predictive models and enable predictions about chemical toxicity using in silico approaches.

1.11.P-We044 Modelling Gene Expression Dynamics During Early Zebrafish Development to Explain Developmental Changes in Detoxification Capacity

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Molecular toxicokinetic-toxicodynamic (mTKTD) models dynamically link exposure to detoxification and lethality via the expression of stress regulator genes (e.g. *cyp1a*, *nrf2*). Currently, these models assume a constant baseline expression for fold-change calculation. However, during early zebrafish embryogenesis, gene expression is highly dynamic. A key event is the maternal-to-zygotic transition (MZT), during which the zygotic genome becomes transcriptionally active and gradually replaces maternally deposited factors. Consequently, stress regulator levels can vary significantly across early developmental stages, implying that the stress response is strongly dependent on exposure timing. Assuming a constant baseline may therefore overestimate the detoxification capacity at stages where stress regulator concentrations are naturally low.

To address this limitation, we developed a set of mechanistic transcription models that capture common expression patterns and incorporate MZT dynamics to generate biologically more profound baselines. Each model describes the total transcript abundance as the sum of maternal and zygotic contributions. Maternal transcripts dominate initially and decay without de-novo transcription, while zygotic transcripts are absent at first but accumulate after zygotic genome activation (ZGA). We explored two ZGA timing mechanisms assuming a maternal threshold or time-dependent activation. After ZGA, transcription either proceeds with zero-order growth or is repressed by a transcription factor. We compared these versions against a commonly used kinetic model.

We fitted and evaluated all models using an RNA sequencing dataset comprising more than 24,000 genes and 18 time points from 0 to 120 hpf using a Bayesian approach. Our results show that 58% of the genes can be described by at least one of the models. For *cyp1a* in particular, the early developmental stages are captured equally well by both the threshold and the timing model. Both models reproduce the initial decline and subsequent increase in transcript levels, whereas the standard kinetic model overestimates the early expression.

The simulated *cyp1a* baseline will be integrated into a recently published mTKTD model (GUTS-RNA-pulse) to assess the impact of exposure timing, comparing effects and detoxification capacity for exposure to diclofenac, diuron and naproxen at 2 hpf (pre-ZGA) and 24 hpf (post-ZGA).

1.11.P-We045 Molecular Insights into Caffeine Toxicity in the Freshwater Gastropod *Physa acuta*
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Caffeine (CAF), a widely consumed psychoactive compound, has recently been recognized as an emerging environmental pollutant in freshwater ecosystems. Previously, it has been shown that relevant concentrations of CAF (5, 30, and 50 µg/L) alter behaviour, including locomotor activity, exploration patterns, and feeding patterns in the freshwater gastropod *Physa acuta*. Building upon these findings, the present study investigates the molecular mechanisms underlying these behavioural effects. Adult snails were exposed to the same CAF concentrations (5, 30, and 50 µg/L) for 24 hours and 7 days. The transcriptional activity of genes associated with the nervous system, as well as the glucocorticoid receptor (Cortisol), known to interact with CAF, was analyzed via RT-PCR. The target genes are: adenosine receptor 1 (A1AR), adenosine A2B receptor (ADORA2B), adenosine receptor A3 (ADORA3), acetylcholinesterase (AChE), glycine receptor subunit alpha 2 (GLRA2), dopamine receptor 1 (DRD1), dopamine receptor 2 (DRD2), serotonin receptor (HTR), ryanodine receptor (RyR), Glutamate Receptor NMDA 2B (GRIN2B), Hydroxysteroid 11-beta-dehydrogenase 1 (HSD11B), FMRFamide receptor (FMRFaR), sodium-dependent noradrenaline transporter (SLC6A2), and GABA Transporter 1 (SLC6A1). Given that the molecular pathways influenced by CAF in aquatic invertebrates remain poorly understood, this research aims to provide valuable insights into the molecular pathways modulated by CAF in freshwater invertebrates, thereby filling this gap. Additionally, the results of this study will contribute to a better understanding of its ecological impact and support environmental risk assessment efforts.

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1.11.P-We046 Measuring Detoxification In-Vivo: Validation of a General Esterase Substrate in Several Freshwater Invertebrates

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General esterase enzymes are a ubiquitous and essential superfamily that mediate neurotransmission and phase I metabolism through cleavage of esterase bonds. They are key for the detoxification of endogenous molecules and xenobiotics and are a promising target to gain mechanistic insight into toxicokinetic processes that could be leveraged to understand (eco)toxicological effects. While the activity of carboxyl/butyryl esterase enzymes has been measured in vitro for a few species-substrate combinations, in vivo assays have not yet been optimized for multiple species, nor validated for several chemical classes. In this study, we present the selection of a fluorogenic substrate for in vivo measurement of general esterase enzyme activity in several aquatic invertebrate species. Basal conversion activity of the substrate 4-methylumbelliferyl butyrate (4-MUB) to 4-methylumbelliferone (4-MU) is measured in vivo by exposure of six freshwater arthropods. The assay conditions are optimized based on the sensitivity of the substrate to pH, light intensity, and temperature. Our findings show that the 4-MUB substrate is mostly stable at physiological pH, in dark conditions and at room temperature. Each species is exposed for 4 hours to a range of 4-MUB concentrations (0 – 104 µM), with solution samples taken every hour. This allows measurement of the formation kinetics of the fluorescent product, 4-MU. The fluorescent units read by the spectrophotometer are converted to pmol/mg protein/hour of 4-MU by measuring the protein content of each species. Preliminary results show low variation in substrate affinity between species. The range of 4-MUB concentrations allowed to select an optimal substrate concentration for each species to be used for the synergism assay. To validate the metabolic activity of 4-MUB, a synergism assay is performed with a known esterase inhibitor, S,S,S-tributyl phosphorothioate (DEF). After assessing the toxicity limits of DEF, the species are pre-exposed to the inhibitor for 24h, and the metabolic activity is assessed with the 4-MUB assay. The results will give insight into the affinity of the substrate with the esterase system of the species. Selection and optimization of substrates like 4-MUB is fundamental to expand the suite of molecular tools in ecotoxicology. These tools help gain insights into the impact of chemical stressors on the detoxification capacity of aquatic invertebrates and into the understanding of toxicity determinants.

1.11.P-We047 Tire Wear Particles as an Oxidative Stressor: Antioxidant Enzyme Response in the Carpenter ants (*Camponotus* spp.)

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Tire wear particles (TWPs) constitute one of the largest sources of microplastic pollution in terrestrial environments, with global estimates at 6.1 million metric tons annually. Unlike conventional microplastics, TWPs represent a complex mixture of synthetic and natural rubber polymers with chemical additives including carbon black, zinc oxide, sulfur, and polycyclic aromatic hydrocarbons. Despite their environmental prevalence, research examining TWP impacts on terrestrial insects remains scarce. This study investigated oxidative stress responses of carpenter worker ants (*Camponotus* spp.) exposed to environmentally relevant TWP concentrations. Ants were maintained under controlled laboratory conditions and assigned to three treatment groups: control, 0.05% TWPs, and 0.50% TWPs, representing TWP levels in roadside soils. TWPs were mixed into the Bhastkar diet and fed to ants over 72 hours. Following exposure, antioxidant enzyme activities, including SOD, CAT, POD, and MDA, were measured. Results demonstrated distinct concentration-dependent oxidative stress patterns. SOD activity exhibited a biphasic response: 0.05% TWP exposure caused 19.4% activation, indicating compensatory antioxidant defense, while 0.50% TWP caused 20.2% suppression, suggesting enzyme system overwhelm. Lipid peroxidation increased dramatically, with MDA levels showing 6.3-fold and 18-fold increases at 0.05% and 0.50% TWP, respectively, indicating severe membrane damage. CAT activity was suppressed by 50% at both concentrations, highlighting catalase inhibition as a critical vulnerability. POD activity showed moderate, non-significant reductions. These findings demonstrate that environmentally relevant TWP concentrations induce severe oxidative stress in carpenter ants, characterized by catalase suppression, extreme lipid peroxidation, and biphasic SOD responses. The magnitude of antioxidant impairment at concentrations found in roadside soils suggests TWPs pose significant biochemical threats to terrestrial invertebrates. Given carpenter ants' keystone roles in forest ecosystems through nutrient cycling and decomposition, TWP pollution may have cascading effects on soil ecosystem functioning. This study provides critical baseline data for understanding TWP ecotoxicological impacts on terrestrial insects and highlights the need for investigation into long-term consequences and specific chemical constituents responsible for oxidative damage.

1.11.P-We048 Applying a Toxicokinetic-receptor Model to Explain Long-lasting Bioaccumulation of Neonicotinoids in Two Invertebrate Species

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Neonicotinoids are an insecticide class whose polarity indicates a low potential for bioaccumulation. However, studies have revealed the tissue concentrations of gammarids to be significantly higher in the field than those derived from laboratory toxicokinetic studies. This demonstrates an environmental risk to non-target aquatic species and further highlights gaps in our understanding of the underlying bioaccumulation processes. An observed elimination resistance of neonicotinoids could be due to the irreversible binding to the nicotinic acetylcholine receptors (nAChRs).

To test this hypothesis, we performed toxicokinetic experiments with three neonicotinoids (i.e., Imidacloprid (IMI), Thiacloprid (THI), and Acetamiprid (ACE)) using two freshwater invertebrate species *Gammarus pulex* and *Hyalella azteca*. We further aimed to elucidate receptor–ligand interactions by examining competition experiments, as these compounds all selectively bind to nAChR but exhibit different affinities and efficacies towards the receptor.

Thus, first, we investigated long-duration kinetics (6 to 18 days) for each compound individually. Subsequently, we conducted sequential exposure experiments, comprising two uptake phases with paired compounds to assess displacement dynamics. 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). Various experiments were used to calibrate and validate a two-compartment toxicokinetic-receptor model, including growth dilution and irreversible receptor binding. The model facilitated the mechanistic understanding of competitive binding at the different subunits of the receptor, explaining neonicotinoid bioaccumulation in aquatic invertebrates.

Altogether, the results emphasize that receptor binding as a cause for bioaccumulation should be considered in chemical risk assessments.

Disclaimer/Disclosure:

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1.11.P-We049 Bayesian Species Sensitivity Distribution Modeling for Microplastics: Integrating Particle Properties and Species-Level Variations of Effect Concentrations

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Because of growing global concern about the environmental impacts of microplastic particles (MPs), scientifically defensible ecological risk assessments are increasingly required. However, such assessments remain challenging due to the diversity of MP properties, such as particle length and shape. In this study, we developed species sensitivity distributions (SSDs) for MPs using hierarchical Bayesian modeling, which accounted for species identity as well as the influence of MP properties on chronic no observed effect concentrations (NOECs). We also incorporated highest observed no-effect concentrations (HONECs) into SSD estimation by appropriately treating them as right-censored data. Using data from a recently updated ecotoxicity database, we analyzed two datasets excluding HONECs (21 species) and including HONECs (33 species). For the HONEC-excluded dataset, the best SSD model, selected based on the widely applicable information criterion, included size category (particle length <83 μm vs. $\geq 83 \mu\text{m}$) and fiber shape, suggesting that smaller particles and fibers were associated with lower chronic NOECs. For the HONEC-included dataset, the best model included particle length and shape (fragment and fiber), indicating that shorter particle lengths and non-spherical shapes were linked to lower chronic NOECs. Median estimates of the hazardous concentration for 5% of species (HC5) ranged from 0.06 $\mu\text{g/L}$ (fiber, <83 μm) to 111 $\mu\text{g/L}$ (non-fiber, $\geq 83 \mu\text{m}$) in the HONEC-excluded dataset. For the HONEC-included dataset, median HC5 estimates ranged from 0.003 to 167 $\mu\text{g/L}$ depending on particle length and shape, with considerable uncertainty in the estimates. Despite uncertainties in modeling, our SSD modeling framework provides a generalizable and data-informed approach to incorporating diverse MP properties and censored effect concentrations to improve ecological risk assessments for MPs as well as other substances.

Disclaimer/Disclosure:

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1.11.P-We050 Trophic Transfer Impact of Combined Microplastics and Tire Particles in Three-Species Freshwater Food Chain

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Microplastics (MPs) and tire particles (TP) are ubiquitous contaminants in aquatic ecosystems, posing complex ecological challenges through their persistence, heterogeneity, and potential for trophic transfer. While previous studies have explored single-species exposures, limited evidence exists on how these mixed polymeric pollutants propagate across multiple trophic levels. This study investigates the trophic transfer, physiological responses, and oxidative stress induced by combined microplastics and tire particles (MPsTP) in a three-species freshwater food chain: *Chlorella vulgaris*, *Daphnia magna*, and zebrafish (*Danio rerio*).

A simplified food-chain model was developed to simulate environmentally relevant exposure (1 mg/L) to MPs, TP, and their mixtures. The MPs comprised polypropylene, polyethylene, and polyethylene terephthalate particles and fibers representing dominant polymers in freshwater environments. *Chlorella vulgaris* cultures were exposed to MPs, TP, and MPsTP mixtures before being fed to *Daphnia magna*, which were subsequently consumed by zebrafish. Fluorescence microscopy confirmed ingestion, retention, and transfer of particles from microalgae to *Daphnia* and ultimately to zebrafish intestinal tissues, demonstrating efficient movement of synthetic particles through the trophic chain.

Biochemical assays revealed that zebrafish exposed via trophic pathways exhibited significantly elevated malondialdehyde (MDA) levels, indicating enhanced lipid peroxidation and oxidative stress, particularly in the MPs and TP groups. Total antioxidant capacity (T-AOC) remained stable, suggesting partial compensation through antioxidant defense mechanisms during short-term exposure. These results demonstrate that MPs and

TP induce sublethal oxidative imbalance at higher trophic levels, even at low concentrations.

This study provides direct experimental evidence that mixed microplastic and tire-derived contaminants are trophically transferred within freshwater ecosystems, eliciting oxidative stress responses in consumers. The findings highlight the mechanistic link between particle ingestion, physiological disruption, and potential ecological consequences, reinforcing the need for integrating trophic-level interactions into predictive ecotoxicology models. Understanding such pathways is crucial for advancing next-generation risk assessment frameworks addressing complex plastic pollution in aquatic food webs.

1.11.P-We051 Modelling the Life Cycle of the Painted Lady Butterfly *Vanessa cardui* Using the Dynamic Energy Budget Theory

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Mechanistic effect models combining Dynamic Energy Budget (DEB) theory with Toxicokinetic-Toxicodynamic (TKTD) approaches offer several advantages. They allow extrapolation from laboratory generated toxicity data to untested scenarios. Time-variable exposure and environmental conditions can be accounted for, and individual-level effects may be extrapolated to the population. In order for toxicity effects observed in the laboratory to be accurately reproduced in the DEB-TKTD model, it is important that the DEB reference model is first able to reproduce the control data sufficiently well. While there are predefined DEB model variants for many groups of organisms that can be fitted to control data (typically composed of literature data and control data from toxicity tests) with relative ease, holometabolous insects represent an underdeveloped group in this regard. The aim of this study was to develop a DEB model structure for the Painted Lady butterfly *Vanessa cardui* that can adequately represent the Lepidopteran life cycle, and to parameterize the model using literature data and newly generated laboratory data.

Building on recently developed DEB models for *Coccinella septempunctata* and *Chrysoperla carnea*, the butterfly's life cycle could be convincingly modeled with only a few modifications. For each new model adjustment, plausible assumptions were implemented separately into the model and compared with the data to make informed model decisions. For example, various assumptions about larval growth behavior were systematically investigated in this manner. Another key aspect was the explicit consideration of the prepupa phase, which was clearly evident in the data as a premature growth stop before pupation. For this purpose, several plausible model assumptions for an adjusted feeding rate, the rate of reserve mobilization, and the energetic investment in maturation during this phase were compared and evaluated. The final model is considered suitable as a reference model for the unstressed organism in DEB-TKTD. Its structure may serve as a reference for other species of Lepidoptera in the future. A DEB-TKTD analysis using this new model is presented in a parallel presentation.

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1.11.P-We052 Linking PBK and DEB models for risk assessments: an example for the wood mouse

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In environmental risk assessment (ERA), laboratory data from test species are used to estimate potential risks of exposures to various species in the field. We present a linked physiologically based kinetics (PBK) and dynamic energy budget (DEB) model that addresses the challenge of extrapolating from laboratory test species to wild species under field conditions.

The PBK component simulates toxicant concentrations across organs and tissues over time, incorporating species-specific physiological parameters to capture interspecies differences in chemical kinetics. We parameterized and validated the PBK model using laboratory rat data for a herbicide, then adapted it for the wood mouse (*Apodemus sylvaticus*) using physiological parameters from the closely related laboratory mouse (*Mus musculus*).

The DEB component simulates effects on organism physiology and was calibrated with wood mouse-specific data on growth and reproduction. By linking PBK predictions of internal exposures to the disruption of

physiological processes in the DEB model, we can capture the herbicide's effects while assuming conservation of internal chemical effects between species.

This integrated PBK-DEB model, when incorporated into the wood mouse population model (APODEMUS), enables the estimation of population-level effects under field-relevant exposure scenarios. Our approach demonstrates how mechanistic modeling can facilitate cross-species extrapolation of exposure and effects beyond standard laboratory test conditions.

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1.11.P-We053 Mechanistic Effect Modeling of Freshwater Mussels: The Role of Life-History in Stress-Driven Population Dynamics

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Assessing stress effects at the population level is crucial for informing ecological risk assessment and science-based management policies. However, determining these effects in laboratory or in-situ settings is challenging due to delayed responses, with impacts often becoming evident only after multiple generations. This is particularly challenging for imperiled taxa experiencing rapid declines driven by environmental and anthropogenic stressors, and whose persistence relies on timely interventions.

Even when individual-level effect data exist for a species of interest or a suitable surrogate, scaling these effects to the population level is not straightforward, and requires consideration of interacting mechanisms and stochasticity. Mechanistic effect models provide a framework to bridge these gaps and translate individual responses – driven by life-history traits – into more realistic population-level effects.

Our research develops a generalized individual-based population model (IBM) for freshwater mussels, a taxon experiencing global decline. This builds upon prior work that combined Dynamic Energy Budget (DEB) and life-history theory to capture sub-lethal individual effects of stressors across three mussel life-history strategies: opportunistic, equilibrium, and periodic.

The modeling approach integrates an individual DEB model into the IBM, parameterized for a representative species of each life-history category, to capture how changes in growth, maturation, and fecundity influence long-term population dynamics. The model is used to simulate a range of impact scenarios and physiological modes of action of a theoretical stressor, while accounting for realistic environmental drivers, inter-individual variability, and intra-specific competition.

Preliminary results show that population-level outcomes differ across life-history categories, even when individual-level responses appear similar. While population decline is evident in most scenarios, opportunistic species seem more resilient due to faster generation turnover, supporting quicker recovery once stress exposure is removed. Equilibrium species may be more vulnerable due to their longer generation times and lower fecundity. We highlight conceptual model, key decisions, structure, and implementation, and present simulation results for selected species. Finally, we discuss the limitations and potential of this framework in supporting ecological risk assessment and guiding conservation strategies.

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1.11.P-We054 Assessing Effects on Lemna Growth under Toxicological Stress in the Funne Water Catchment

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Understanding how plant protection products affect aquatic ecosystems remains a major challenge in environmental risk assessment. Landscape-scale modelling that integrates hydrological, exposure, and ecological processes offers one of the most advanced ways to refine surface water risk evaluations.

In this study, the toxicokinetic–toxicodynamic model for *Lemna* species within the xLandscape framework, originally based on the SETAC approach implemented in the CVAI package, was extended to account for water flow velocity as a growth-limiting factor influencing biomass dynamics. A sensitivity analysis has been conducted for nutrient availability of phosphorus and nitrogen, showing that both parameters are non-limiting factors due to increased level in surface waters in agricultural landscapes. The model was then applied and tested to capture how hydraulic conditions shape *Lemna* growth under toxicological stress at the catchment scale. Model simulations were conducted across the 54 km² Funne catchment in North Rhine-Westphalia, Germany, representing an intensively managed agricultural landscape. The modelling scenario was parameterized using monitoring data on product applications, land use, hydrography and hydrology. Derived landscape-scale EP50 values, representing the exposure reduction factor leading to a 50% growth inhibition, were evaluated across different application and management patterns improving our understanding of spatially distributed aquatic effects and their implications for regulatory risk assessment considering ecological habitat parameter such as flow velocity.

1.11.P-We055 Suitability of Delta log D to Predict pH-dependent Toxicity Differences Across Aquatic Organism Groups for Ionisable Substances

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Depending on ambient pH, ionisable substances occur as mixtures of neutral and ionic microspecies that differ in membrane permeability and thus in bioavailability and toxicity. In fish embryo acute toxicity tests, previous studies have evaluated the difference in the distribution coefficient log D between two pH conditions (i.e. delta log D) as descriptor of membrane passage and a predictor of pH-dependent changes in toxicity using the partitioning coefficient for octanol-water as a descriptor for the trans-membrane diffusion capability of organic chemicals. Although literature data for other organism groups indicate higher toxicity of the neutral form, the transferability of this simple delta log D model beyond fish embryos has not been systematically assessed. In this work, we compile published aquatic toxicity data for ionisable substances across several organism groups and pH conditions and derive delta log D for each test substance and pH combination. We analyse how well delta log D explains observed pH-specific differences in toxicity across taxa and substances. This provides a first quantitative insight into the applicability and limitations of the delta log D approach for predicting pH-dependent toxicity differences across aquatic organism groups.

To facilitate the consideration of pH-dependent toxicity in a regulatory context, we also present an open-source tool that models and visualises log D–pH relationships for monoprotic acids and bases based on experimentally derived log D values at defined pHs. This supports the use of delta log D as a simple, transparent predictor in extrapolating laboratory toxicity data to variable field conditions and may therefore help align risk assessments with environmental protection goals.

1.11.P-We056 Predicting Chemical Risks in a Changing Climate Through Model-Guided Study Designs.

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Evaluating toxic effects is critical for chemical risk assessment. However, additional climate-change-induced stressors and dynamic exposure conditions are making this complicated. To meet this challenge, regulators are turning to predictive, mechanistic models, such as General Unified Threshold models of Survival (GUTS). However, laboratory toxicity tests have traditionally been designed for hypothesis testing or dose-response analyses, resulting in data that are not ideally suited to dynamic, multi-stress modelling. We aim to provide data- and theory-driven evidence on how to optimise ecotoxicology study designs to make them “model-ready”.

First, we identify the defining features of an optimal experimental design for dose-response and GUTS modelling. 216 time-resolved toxicity test datasets, complemented by meta-data from their source papers, are used to train a machine learning model. This enables us to identify which design choices (e.g., concentrations and replicates) predict more accurate and precise toxicity estimates (e.g., high goodness-of-fit and low coefficients of variation for the lethal concentration LC50). This will be compared with a theoretical D-optimality framework. We hypothesize that designs prioritizing concentrations over replicates will yield more robust data for modelling.

Second, we quantify the impact of design choices on GUTS model reliability. We conduct a sensitivity analysis selecting the most robust datasets and systematically subsampling them to mimic poorer designs. This includes a "Regulatory Test" in which the data is thinned to meet the minimum requirements of the OECD guidelines, and allow results of poorer designs to be interpreted while considering their experimental uncertainty.

Third, we demonstrate the real-world consequences of this uncertainty. GUTS models parametrised from both full- and thinned-datasets will be challenged using synthetic, time-varying scenarios that combine chemical exposure and climate stressors. We predict large uncertainty for the thinned-data model and more accurate and precise predictions for the full-data model.

This project produces a portfolio of recommendations that comply with the FAIR principles (Findable, Accessible, Interoperable, and Reusable) to help regulators and scientists in adapting experimental protocols, supporting the 3Rs approach (Refinement, Reduction, and Replacement) and strengthening the scientific basis for using predictive models in a changing climate.

1.11.P-We057 How Species Sensitivity Variation and Biotic Interactions Buffer Food Web Endpoints under Chemical Exposure

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Chemical pollution in aquatic ecosystems is an escalating global concern, yet our understanding of how complex networks of species interactions combine with variation in species sensitivities to determine community-level responses to chemical stress remains limited. In this study, we asked how species interactions and sensitivity determine biodiversity dynamics and community biomass. We constructed synthetic food webs that varied in their biotic interaction structure and complexity, and assigned empirically grounded patterns of sensitivity variation across multiple chemical target groups. We then simulated food web responses to sequential chemical perturbations and fitted shape constrained additive models to estimate three endpoint levels (5%, 50%, and 100% loss) for species persistence (biodiversity) and community biomass (ecosystem function). We found that the proportion of links among species (connectance) and maximum trophic level exert strong effects on both persistence and biomass at the 5% and 50% endpoints. Highly connected food webs tended to buffer early stage losses to chemicals, whereas higher maximum trophic levels were correlated with lower biodiversity and biomass under chemical stress, likely due to stronger trophic cascades. In contrast, variation in species sensitivities played a buffering role at the 100% endpoint compared to the 5% and 50% endpoints. Variation in species response to chemical stress (response diversity) substantially buffers against complete food web collapse. Together, these findings highlight the distinct roles of species-level sensitivity and food web structure in shaping biodiversity and biomass responses to chemical pollution.

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1.11.P-We058 From Individual Traits to Food Web Effects: Predicting Contaminant-induced Impact on Theoretical Aquatic Food Webs Using Allometric Scaling

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Chemical contaminants, stemming from human activities, are omnipresent in aquatic systems nowadays. Among many adverse effects, these chemicals may alter the body sizes of organisms. This may have important indirect effects on species interactions, as many predator-prey interactions are driven by body size differences. Therefore, such a change in an individual trait can indirectly affect the functioning of the entire food web. To get a better understanding of how these chemicals affect food-web properties, we first performed a meta-analysis and then simulated food-web models based on this meta-analysis.

After reviewing 3586 papers, we selected 38 that measured the body length and weight of aquatic organisms under exposure to different chemicals. We then derived a simple model on how chemicals affect the body size of species. Lastly, we used a trait-based food web model to simulate the effects of increased chemicals and ultimately assess their impact on the functioning of the entire food web.

We hypothesize that 1. increasing the concentration of a particular substance leads to changes in body weight and length. 2. These changes in body size affect species interactions and will hence affect the entire community

3. The most sensitive species are not necessarily the species most affected by the chemicals, but rather those that depend on the structure of the food web.

1.11.P-We059 Exploring Pesticide Effects on Biological Pest Control Through Ecological Modelling

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The prospective environmental risk assessment of pesticides is shifting toward approaches that explicitly protect biodiversity and ecosystem services. Regulation of arthropod pests is one such service, with *Orius laevigatus* being a service providing unit for greenhouses and field crops. It preys on the western flower thrips, *Frankliniella occidentalis*, which rapidly develops insecticide resistance. Understanding the effects of pesticides on *O. laevigatus* is crucial for protecting this non-target species and maintaining biological control. Some earlier studies showed that pesticides reduce pest numbers only when natural enemies are absent. In modelling experiments, predators largely determine pest population levels, and increased predator mortality can increase pest densities. Whether these findings hold under higher ecological realism, with varying environmental conditions, and longer time scales, remains uncertain. This work explores the impact of insecticides on biological pest control under more realistic environmental conditions using an agent-based predator–prey model. The model represents *O. laevigatus* as individuals at different life stages with individual-based properties. Thrips are included as super-individuals to quantify pest control. Model processes include development, reproduction, dispersal, overwintering, and mortality. Predator–prey interactions include feeding by nymphs and adults. Pesticide effects are implemented using toxicokinetic-toxicodynamic modelling with a selected insecticide. Sensitivity analysis will examine how variation in parameter ranges influences predator and prey densities. All processes occur in a homogeneous space at 1 m² resolution and a time step of one day. We hypothesize that the results will be consistent with earlier studies: insecticide applications will either increase or not significantly change average pest densities when natural enemies experience additional mortality. Incorporating temperature dependence of processes and toxicity over time is likely to produce more context-dependent interactions and emergent behaviours, such as delayed pest outbreaks, altered seasonal population structure, and variable overwintering success. This work supports more ecologically grounded predictions of how pesticides influence biological pest control and will further provide a tool for evaluating impacts on the non-target arthropod species, helping the shift towards a more realistic environmental risk assessment.

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1.12 In Vitro Tools and Biosensors for Assessing the Mixture Effects of Contaminants of Emerging Concern in Water

1.12.T-01 The Key Factor Toxicity: Development and Application of a Tool to Characterize the Toxic Pressure of Chemical Pollution in Surface and Drinking Water

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Understanding the effects of chemical mixtures in surface waters is crucial for protecting ecosystems and drinking water quality. Thousands of substances enter aquatic systems via wastewater, agriculture, and industry, forming complex mixtures whose combined effects are poorly addressed by single-compound monitoring. This knowledge gap limits risk prediction for ecology and human health.

To address this challenge, we developed the Key Factor Toxicity (KFTox) Instrument and webtool, integrating two complementary approaches: a Chemistry Track and a Bioassay Track. The Chemistry Track calculates mixture toxic pressure as multi-substance potentially affected fraction (msPAF) using species sensitivity distributions (SSD) of individual chemically monitored compounds and summarizes the results into five categories. The Bioassay Track measures biological activity through a panel of in vitro and in vivo assays, benchmarking the measured activities against effect-based trigger values for ecological and health relevance.

Both tracks provide intuitive, color-coded outputs.

A nine-month case study included surface water abstraction points, process water, and produced drinking water. Chemical monitoring covered >500 parameters; eight bioassays targeted general toxicity, endocrine disruption, oxidative stress, and receptor-mediated effects. The Chemistry Track indicated low toxic pressures, while the Bioassay Track revealed multiple activities exceeding trigger values—indicating possible risks not captured by the Chemistry Track. Each bioassay added unique, relevant information despite a deliberately limited set.

The tracks proved complementary: the Bioassay Track e.g. detected high AhR-mediated activity despite negligible PAH msPAF in the Chemistry Track, suggesting unmonitored AhR-agonists. Conversely, the Chemistry Track included metals and nutrients beyond the scope of the Bioassay Track. Combining both approaches therefore ensures a more representative water quality assessment.

The KFTox instrument offers a robust, integrated framework for assessing chemical water quality. It provides a practical framework for assessing toxic pressure in water systems and to prioritize mitigation measures, supporting the objectives of the EU Water Framework Directive.

1.12.T-02 A Pan-European Bioassay-Based Assessment of Endocrine Activities Across Multiple Environmental Compartments

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Endocrine-disrupting compounds (EDCs) encompass a broad range of chemicals from diverse sources and remain a major challenge for environmental monitoring when using conventional target chemical analysis. In vitro bioassays provide a powerful complementary approach by detecting the endocrine activities, although they cannot identify causative substances. Within the Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC), an integrative approach of chemical analyses and a broad battery of in vitro bioassays has been applied to comprehensively characterize EDC contamination across multiple environmental matrices (surface water, air, and soil) in Europe.

In 2023-2024, coordinated sampling campaigns were conducted covering all European regions and diverse anthropogenic pressures (urban, industrial, agricultural, etc.). A total of 117 water, 42 soil, and 19 air samples were collected and processed using standardized extraction protocols. Water and soil extracts were analyzed with in vitro bioassays targeting nine human nuclear receptors, i.e., the Androgen Receptor (AR), Estrogen Receptor (ER), Glucocorticoid Receptor (GR), Mineralocorticoid Receptor (MR), Progesterone Receptor (PR), Aryl Hydrocarbon Receptor (AhR), Pregnane X Receptor (PXR), Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ), and Retinoic Acid Receptor- α (RAR α), as well as the competition with thyroid hormone to bind its transport protein, Transthyretin (TTR). Air extracts were tested for AR, ER, AhR, PXR, and TTR. In water samples, fish receptors for selected endocrine modes of action (AR, MR, PR, AhR, PXR) were also included to explore potential interspecies differences. Defined quality assurance and control measures were implemented throughout sampling, extraction, and bioassay procedures.

Agonistic endocrine activities are widespread across surface water samples, following the order ER >> AR > PR, GR, MR, RAR α . TTR, PPAR γ , and PXR were detected at >80% of sites. Western and Eastern Europe showed the strongest responses, with some samples exceeding the proposed effect-based trigger values for ER and TTR. No clear link with anthropogenic pressures was found. Next steps include data interpretation for air and soil, and comparison with data from chemical analyses.

Overall, this study represents a large-scale pilot investigation of EDC contamination across multiple environmental compartments in Europe using an extensive battery of bioassays and novel modes of action.

1.12.T-03 Getting a Grip on Pesticide Mixture Toxicity Using Effect-Based Methods

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Pesticides are widely used to enhance crop productivity, but their application leads to emissions that form complex mixtures in surface waters, posing risks to aquatic ecosystems. Effect-based methods, which capture the combined biological activity of chemical mixtures, offer a more realistic assessment of ecotoxicological hazards than evaluations of single substances. This study aimed to examine the suitability of various effect-based methods for assessing the toxicity of representative pesticide mixtures. Four mixtures (herbicides, insecticides, fungicides, and azoles) were formulated based on their occurrence in Frisian agricultural drainage ditches where pesticide levels frequently exceed environmental standards. Compounds were combined at their EC50 or at the highest feasible concentrations due to solubility constraints. Chemical analyses were performed to confirm stock solution concentrations. A battery of in vivo bioassays (algae, *Daphnia*, bacteria) and in vitro CALUX assays covering cytotoxicity, endocrine and metabolic disruption, oxidative stress, and genotoxicity were used to evaluate the potential toxicity of the mixtures. All in vivo assays showed clear effects. Insecticides were most toxic to *Daphnia* and bacteria, while herbicides caused strong algal responses, reflecting the expected sensitivity of these organism groups. Fungicides and azoles also induced substantial in vivo toxicity, even though their modes of action are not directed at the primary target organisms in the assays used, indicating non-target toxicity. The PXR, anti-AR, and anti-PR in vitro assays were the only CALUX assays that responded to the fungicide and azole mixtures. Herbicides and insecticides did not trigger activity in the applied in vitro assays. These findings highlight that fungicides may drive observed toxicity in the field and that current environmental risk assessment lacks an in vivo fungal bioassay to adequately capture their ecological impact. A multispecies fungal bioassay may therefore provide a valuable addition to future effect-based monitoring strategies. Here, we showed that effect-based methods are well-suited for assessing the toxicity of pesticide mixtures and represent a valuable tool in environmental monitoring. Toxic pressure from the fungicide and azole mixtures is captured by these methods, although a comprehensive risk assessment of fungicides requires an in vivo fungal bioassay to accurately reflect their ecological impact.

1.12.T-04 High-Throughput Receptor-Based Bioassay For Non-targeted Screening of Bioactive Contaminants in Water

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Biologically active micropollutants, including pharmaceuticals, hormone disruptors, and pesticides are increasingly detected in surface waters worldwide. Psychoactive pharmaceuticals are one of the most frequently detected group of water pollutants. Their persistence, low-dose bioactivity, and potential biotransformation raise concerns for aquatic ecosystems and human health. Their risk to the aquatic environment is generally assessed by a combination of chemical and biological analyses. Yet, these methods are often resource-intensive and time-consuming in practical application. As such, there is a need for a rapid, sensitive, and biologically relevant method capable of screening complex water matrices for a broad spectrum of bioactive compounds. One such innovation is multiplex receptor technology called “receptomics” developed by Wageningen University and Research. This platform uses a flow-cell sensor system to assess dozens of G-protein-coupled receptors (GPCR) activation in response to contaminants in water samples that acting like receptor’s ligands. Receptor’s activation provides a unique biological activity fingerprint enabling holistic assessment of bioactive contaminants (www.receptomics.com). The broad diversity of GPCRs allows screening pharmaceuticals in water samples. The receptor repertoire might be extended to include nuclear receptors or non-human GPCRs, namely, insect or zebrafish’s GPCR, increasing environmental relevance. The assay allows repeated measurements every five minutes, enabling rapid screening of both pure compounds and complex mixtures. As a proof-of-concept a psychoactive pharmaceutical was tested on a set of serotonin and muscarinic receptors. Already at 0.32 µM of the psychoactive drug- clozapine activated several muscarinic receptors. Although the tested concentrations exceed typical environmental levels, these results establish the biosensor’s capability to detect receptor-specific activity patterns of psychoactive pharmaceuticals, confirming that the platform can measure reproducible and biologically meaningful GPCR activation. The system is a sensitive, rapid, and high-throughput platform that can support water managers and drinking water companies to monitor water quality continuously. The developed system can also be used as an early warning system and as a screening tool to develop risk-driven measurement programs in the (drinking) water sector.

1.12.T-05 Combining Sample Fractionation and Pull-Down Assays for Identifying Thyroid Hormone Disruptors in Non-Targeted Screening of Complex WWTP Effluents

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Micropollutants discharged from wastewater treatment plants continue to pose a significant challenge, as complex mixtures of chemicals can disrupt sensitive biological pathways, such as those involved in thyroid hormone transport. Transthyretin (TTR) is a key carrier of thyroxine (T4), and many environmental contaminants can interfere with TTR-T4 binding. Identifying the specific drivers of this disruption is challenging because environmental extracts contain thousands of co-eluting compounds that complicate non-targeted screening (NTS). Here, we combined effect directed analysis with an affinity-based pull-down assay to improve the identification of TTR-binding ligands in passive sampler extracts from a large municipal WWTP. Passive samplers deployed for 16 weeks provided time-integrated extracts, which showed strong TTR-binding inhibition. Primary HPLC fractionation distributed activity mainly into fractions F7 and F8 from the eleven collected ones. Secondary fractionation of these fractions again revealed broad activity across multiple sub-fractions, indicating contributions from many compounds with diverse physicochemical properties. While fractionation reduced sample complexity, activity remained widely distributed, limiting its ability to isolate causal chemicals.

To selectively capture TTR ligands, the original extract and active fractions were analyzed using a pull-down assay with His-tagged TTR. Non-targeted screening of eluates enabled the identification of 47 compounds with confirmed structures and demonstrated bioactivity. Several compounds were identified as novel environmental TTR ligands, while known ligands including diclofenac, LAS, and benzophenone-3 were also detected. Quantification and mass-balance evaluation showed that identified ligands explained only a minor portion of the observed activity, since number of features remained unidentified due to gaps in spectral libraries. Our study demonstrates that fractionation alone can be insufficient for resolving complex wastewater extracts, whereas the pull-down assay reliably enriches active ligands and significantly enhances identification. Combining both approaches provides a powerful strategy for uncovering thyroid-disrupting chemicals in environmental mixtures.

Disclaimer/Disclosure:

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1.12.P In Vitro Tools and Biosensors for Assessing the Mixture Effects of Contaminants of Emerging Concern in Water

1.12.P-Mo066 Monitoring Endocrine Activities of Surface Waters Using Transgenic Zebrafish-Based Bioassays

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Over the past decades, numerous studies have reported the contamination of aquatic environments by endocrine-disrupting chemicals (EDCs), resulting in adverse health effects on sensitive aquatic species. Monitoring environmental endocrine activities has thus become increasingly important for assessing the quality of water bodies. In a previous study, we demonstrated the relevance of the EASZY assay (OECD TG250), which uses tg(cyp19a1b:GFP) zebrafish embryos, to quantify in vivo estrogenic activity (ER) in water samples collected across Europe. Estrogenic activities were correlated with in vitro responses and with EQS mixture risk assessment. Although relevant, the EASZY assay remains limited to ER-mediated activity. To refine EDC monitoring in water samples, the EASZY test design was expanded to include assessment of gene expressions, enzymatic activities regulated by other receptor signaling pathways such as Aryl Hydrocarbon Receptor (AhR), Glucocorticoid Receptor (GR), and Pregnane X Receptor (PXR). In addition, a novel zebrafish-based assay, EMERGE, using tg-(cyp3a65:GFP) zebrafish embryos, was applied to assess the effects of aquatic contaminants on intestinal expression of cyp3a65, a multi-receptor-regulated gene involved in the endogenous and xenobiotic metabolism. Twenty-six surface water samples were collected at the French national scale within the framework of the Horizon EU PARC project, and their organic extracts were tested at different relative enrichment factors (REF) in each bioassay.

Estrogenic activity in several surface waters was evidenced through their capacity to induce GFP in EASZY, in agreement with in vitro ER activities measured at the same sites. Weak dioxin-like AhR-mediated activities were also observed in some samples through their capacity to induce cyp1a expression and/or CYP1A activities. Notably, many environmental samples were active in EMERGE, leading to concentration-dependent inductions in intestinal GFP fluorescence driven by the cyp3a65 promoter, at levels close or similar to those of the positive control.

Overall, this study demonstrates that multiple endocrine activities can be monitored using zebrafish-embryo based-assays, enabling quantification of multi-endocrine activities and the identification of contaminated and hotspot sites. Further, the in vivo data will be compared to endocrine activities measured in vitro and chemical analysis of environmental contaminants measured at the same sites.

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1.12.P-Mo067 Benchmarking Historical Data of Estrogenic Activity of Waters From Rio de Janeiro State Against Effect-based Trigger Values

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The occurrence of estrogenic micropollutants in aquatic ecosystems is a widespread issue affecting societies across the globe. Considering the challenge of quantifying these substances at low concentrations, in vitro bioassays are well-established bioanalytical tools capable of capturing mixture effects in complex samples and thus complement chemical analysis in environmental monitoring. In the present study, historical data of estrogenic activity of several water matrices from Rio de Janeiro state, assessed using the Yeast Estrogen Screen, were compiled and benchmarked against proposed effect-based trigger (EBT) values. It included large rivers (n=55), urban streams (n=12), coastal lagoons (n=54), bay/sound waters (n=10), and tap waters (n=21) to a total of 152 samples investigated over the last decade. The contribution of suspended particulate matter (SPM) was also evaluated in selected surface waters. Estrogenic activity was very often (> 75%) detected in streams, bay/sound waters, lagoons and rivers, with estradiol-equivalents (EEQ) quantified in the ranges 3.47 – 189 ngE2/L, 8.96 – 76.9 ngE2/L, 1.49 – 65.4 ngE2/L and 0.28 – 82.9 ngE2/L, respectively, but was rarely observed in tap waters (9.5%, EEQ: 1.47 – 3.49 ngE2/L). Although the estrogenic activity of assessed tap waters was never below the proposed threshold for potable use of 12 ngE2/L in the YES assay, the various surface waters usually exceeded a calculated EBT value of 1,17. Moreover, the median concentration in coastal lagoons was around 10xEBT, whereas median concentrations in way waters and urban streams ranged between 10 times and 100 times higher than the EBT value, highlighting the elevated risk for the aquatic ecosystems. The fraction of EEQ associated with SPM in surface waters ranged from 10% and 90%, which shows a great contribution to the total estrogenic activity. Nevertheless, all samples evaluated for SPM already exceeded the EBT value through their dissolved fraction alone, suggesting that this assessment approach might be protective. The results reinforce a worrisome panorama for the investigated aquatic environments, especially in lentic and receiving waters.

1.12.P-Mo068 The Amphipod Ecdysone Receptor (EcR): New in vitro Approach for the assessment of arthropod endocrine disrupting chemicals and water biomonitoring

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The ubiquitous presence of endocrine-disrupting chemicals (EDCs) in aquatic environments threatens wildlife, food webs, and water quality. By disrupting endocrine signaling pathways, these substances may impair the development, reproduction, and overall health of exposed organisms. Nuclear receptors (NRs) are a family of transcription factors, key in endocrine regulation, which are often ligand-induced and, thus, vulnerable to EDC effects. This susceptibility has guided the development of active molecules targeting specific NR-mediated pathways. However, lack of proper hazard characterization can put off-target species vulnerable to the action of EDCs and contaminants of emerging concern (CECs). The ecdysone receptor (EcR) is an excellent case study. The EcR alongside the retinoid x receptor (RXR) form a heterodimeric complex initially assumed to be

arthropod exclusive. This apparent group specificity has prompted the design of pest-control chemicals acting on ecdysone-induced pathways (e.g., insecticides). Yet, the elucidation of a broader EcR phylogenetic distribution may extend the action of such substances to off-target species. While vertebrate NR-based reporter gene assays (RGAs) are currently included in environmental risk assessment (ERA) frameworks (e.g., OECD, EPA, WFD), a striking knowledge gap still exists regarding NR-based assays for environmentally relevant invertebrate species, such as amphipods commonly used in biomonitoring. In this work, we address this gap by focusing on two ecdysone-related NRs (EcR and RXR) of the keystone amphipods *Gammarus fossarum* and *G. locusta*. Using luciferase-based assays, we characterized EcR:RXR function and screened putative EDCs. This approach allowed the identification of chemicals with potential EcR-modulating activity (e.g., diacylhydrazines). Additionally, to address the elusiveness of EDCs and mixture effects – key challenges in ERA – we further applied this mechanism-based approach to environmental extracts. For this, eighteen surface water samples from the French Water Agency's Rhône Méditerranée Corse monitoring network were processed by solid-phase extraction to obtain natural mixtures, among which four sites displayed EcR-agonistic activity. To our knowledge, this work provides the first proof of concept for in vitro detection of EcR-modulatory activity in natural water samples, aiming to improve current biomonitoring strategies.

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1.12.P-Mo069 A bioanalytical and chemical approach for wastewater discharge: beyond detected chemicals for water quality assessment

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Targeted chemical analysis is widely used for water quality monitoring but has significant limitations, as it cannot detect unknown chemicals or account for mixture effects. To address these gaps, applying effect-based methods (EBM) are gaining interest. Here, we applied a battery of in vitro bioassays to wastewater influent and effluent from four wastewater treatment plants (WWTPs) in Victoria, Australia, as well as surface water upstream and downstream of the WWTPs, to assess the potential risks from mixture effects. The bioassays were complemented with targeted chemical analysis of over 600 chemicals. Our study shows that both the chemical and bioanalysis had higher chemical concentrations and effects in discharged effluent compared to surface waters, confirming WWTPs as key pollution sources. Though most WWTPs were able to remove over 80% of bacterial toxicity, photosystem II inhibition, estrogenic activity and oxidative stress response, one site had poor removal of estrogenic and aryl hydrocarbon (AhR) activity. This site also had a lower median chemical removal efficiency (40%) compared to the other WWTPs (72-88%). Surface water samples downstream of some of the WWTPs exceeded ecological effects-based trigger values (ecoEBTs), suggesting effluent discharge contributing to increased estrogenic and AhR activity, as well as oxidative stress. Despite the large number of detected chemicals, only a small fraction of the observed biological effects could be explained using iceberg modelling, highlighting the presence of unknown bioactive contaminants. These findings underscore the need for integrated chemical analysis with EBM to accurately assess water quality, and improve protection of aquatic ecosystems, worldwide.

1.12.P-Mo070 In Vitro Assessment of Chlorine and Monochloramine Reactivity During Gastrointestinal Digestion

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Nowadays, disinfectants such as chlorine (HOCl) or monochloramine (NH₂Cl) are used to treat drinking water along the distribution network. However, little information is available on the impact of those oxidants on the gastrointestinal tract (GI). Here, we examine how they interact with digestive fluids along the GI, their degradation pathways and their potential to form new disinfection by-products (DBP) using an in vitro digestion protocol.

The simulation of treated water ingestion is performed using the INFOGEST in vitro model. This standardised method simulates salivary, gastric and intestinal digestion, using digestive enzymes: α -amylase, pepsin and pancreatin. In order to study the evolution of residual disinfectant concentrations during these phases, oxidant concentrations were quantified using the N,N-diethyl-p-phenylenediamine method after each stage. The results obtained were compared with kinetic simulations. Oxidant-digestive enzyme interactions were studied by HPLC-DAD/Fluorescence and the by-products formed by GC-MS/MS. An AGS (stomach) culture and a coculture of Caco2/HT29-MTX (intestine) were used to study toxicity of the DBP formed after digestion on both organs.

Findings show that after ingesting chlorinated water, sodium hypochlorite reacts with salivary ammonium to produce monochloramine, which partly oxidizes α -amylase. During gastric digestion, monochloramine further reacts with pepsin. Both HOCl and NH₂Cl are consumed by these interactions, leaving no detectable oxidant residuals after two hours. HPLC analysis confirmed these enzyme-oxidant interactions with different reaction rates, slower for NH₂Cl. As a result, active disinfectants rarely reach the intestine directly.

The implication is that while residual disinfectants are neutralized before reaching the intestine, their transformation products may pose a greater toxicological risk than the oxidants themselves. Ongoing work includes identifying these DBPs and determining their toxicity, as well as assessing if ingestion causes oxidative stress or inflammation in digestive cell cultures. It is important to note that such in vitro simulations simplify reality and do not account for factors like the gut microbiota or certain serum proteins. Nonetheless, this approach provides an initial framework for understanding the health impacts of drinking water disinfectants and their by-products.

1.12.P-Mo071 Thyroid hormone-Disrupting Potential of Wastewater Treatment Plant Effluents Across Europe: Insights from an In Vitro Bioassay Battery

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Growing concern over contaminants in freshwater ecosystems requires advanced analytical techniques for their detection and quantification. Wastewater treatment plant (WWTP) effluents are a major source of endocrine-disrupting chemicals (EDCs), which can interfere with thyroid hormone regulation.

In this study, effluents from 20 WWTPs of varying capacities and employing different treatment technologies across 15 European countries were collected using on-site large-volume solid-phase extraction. The concentrated extracts were assessed using an in vitro bioassay battery targeting key mechanisms of thyroid hormone disruption (THD). The tested endpoints included sodium/iodide symporter (NIS) inhibition, thyroid peroxidase (TPO) inhibition, transthyretin (TTR) binding competition, thyroid receptor (TR) agonism and antagonism, as well as activation of the aryl hydrocarbon receptor (AhR), which is known to interact with thyroid hormone homeostasis.

Our results show that several WWTP effluents from different countries exhibited effects across multiple THD mechanisms-. Particularly pronounced activity was observed for the inhibition of, TPO and TTR binding assays, accompanied by AhR activation in many samples. These findings indicate that compounds with THD potential are widely present in treated wastewater across Europe. To assess potential drivers of the observed effects, we applied iceberg modeling combining measured target compound concentrations with their known effect potencies from the literature. Although a number of individual chemicals could be linked to parts of the observed bioactivities, the majority of the effects remained unexplained, indicating that substantial contributions likely stem from compounds outside the target list or from mixture interactions.

Overall, this study provides important new insights into the THD potential of WWTP effluents at the European scale and demonstrates the value of integrating bioassays with chemical analytical tools. The presence of EDCs capable of interfering with thyroid hormone regulation in treated wastewater emphasizes the need for more comprehensive monitoring frameworks and regulatory measures to better assess, manage, and mitigate environmental contamination.

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1.12.P-Mo072 Assessing the Impact of Quaternary treatment at a Municipal Wastewater Treatment Plant on Surface Water Quality through a Multi-Level Evidence Approach.

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Urban wastewater treatment plants (UWWTPs) in Flanders treat 88% of domestic wastewater, primarily targeting organic matter and nutrients. Yet residual micropollutants remain a challenge for achieving good chemical and ecological status under the Water Framework Directive. The revised Urban Wastewater Treatment Directive (2024/3019) now requires large UWWTPs and UWWTP's in sensitive areas to implement quaternary treatment for micropollutant removal in order to improve water quality in Europe.

In April 2025, the first full-scale quaternary treatment step in Flanders was commissioned at the Aartselaar UWWTP, which discharges into a small watercourse where effluent contributes approximately 50% of the river. The advanced treatment combines ozonation and granular activated carbon (GAC), both contributing to the reduction of micropollutants and associated toxic effects, with the extent of removal depending on operational conditions.

Aquaflin NV and the Flanders Environment Agency assess treatment efficiency and improvements in surface water quality through a multi-level evidence monitoring approach. Monitoring occurs at the UWWTP and in the receiving water upstream and downstream, enabling evaluation of both removal performance and ecological impact. Chemical analyses target a broad spectrum of micropollutants, including pharmaceuticals and pesticides. In addition to chemical monitoring, effect-based methods combining *in vitro* approaches (using CALUX bioassays) and *in vivo* approaches are applied to capture cumulative biological responses.

A reference monitoring phase prior to commissioning revealed that effluent discharge significantly affected downstream water quality, with increased pharmaceutical concentrations and endocrine-disrupting effects. With quaternary treatment now operational, first observations indicate that negative impacts identified during the reference phase can be reversed, supporting the role of advanced treatment in improving ecological water quality.

Disclaimer/Disclosure:

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1.12.P-Mo073 From gene to behaviour: combined exposure of Mg-Al layered double hydroxide and Epirubicin on *Danio rerio* larvae

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Advanced technologies and drugs has led to an increase in emerging contaminants, including nanomaterials like layered double hydroxides (LDH) and antineoplastic agents (AA). LDH are 2D engineered nanomaterials with versatile properties, enabling their use in diverse fields (e.g., corrosion protection; environmental remediation). AA affects aquatic organisms at low concentrations and has been frequently detected in water bodies. LDH, as a drug vehicle for AA, may co-occur in aquatic ecosystems through contamination pathways. However, information regarding their single and combined effects remains limited. Our study evaluated the single and combined toxicity of Mg-Al LDH and epirubicin in zebrafish, assessing multiple endpoints, including embryo development, larval behaviour, DNA damage, biochemical changes, and alterations in gene expression. Single exposure to Mg-Al LDH (13-100 mg/L) caused no mortality or morphological alterations, indicating low acute toxicity. Epirubicin exposure resulted in an LC50=49.3 mg/L and a NOEC for malformations <11.5 mg/L. Mixtures showed no significant change in LC50 values with increasing LDH concentrations, suggesting LDH neither enhanced nor mitigated epirubicin acute toxicity. Epirubicin and M1–M2 mixtures (low LDH with the lowest and highest epirubicin concentrations) significantly increased catalase activity, reflecting activation of antioxidant defence, while mixtures with higher LDH concentration showed no significant effects. No

significant effects were detected for other biomarkers or locomotor behaviour. LDH caused no DNA damage, while epirubicin induced genotoxic effects at 92 and 206 µg/L. When combined with LDH (M1-M5), Tail DNA (%) remained <10%, suggesting LDH influence on epirubicin genotoxicity through adsorption, as confirmed by chromatographic analysis in the tested media. Gene expression analysis showed 100 mg LDH/L induced no change in tp53, catalase, or tbx5a, while 500 µg epirubicin/L caused downregulation of tp53 and catalase, consistent with oxidative stress. These patterns under mixed exposure support that LDH interacts with epirubicin, reducing its bioavailability through likely surface adsorption, thereby antagonizing its genotoxic effects. Overall, epirubicin exerted significant effects on zebrafish, while LDH showed low toxicity and reduced epirubicin effects when co-exposed, highlighting the importance of analysing combined exposures for environmental risk assessment of nanomaterials and drugs.

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1.12.P-Mo074 Embedding Effect-Based Methods in Regulatory Water Quality Assessment: Dutch Practice and European perspective

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Bioassays, or effect-based methods (EBMs), directly measure biological activity caused by chemicals in water samples, capturing combined effects of known and unknown substances. EBMs complement chemical analysis and provide a more comprehensive assessment of water quality.

This work explores practical integration of EBMs into two key European legislative frameworks: the Drinking Water Directive (DWD) and the Water Framework Directive (WFD). It discusses examples of current applications in the Netherlands and the position of EBMs in Europe.

The current DWD, effective since 2023 (EU 2020/2184), introduced a risk-based approach (RBA) requesting monitoring (and subsequent risk management) with focus on emerging and catchment-specific contaminants to ensure that drinking water treatment sufficiently protects human health against harmful chemical effects. While EBMs are not explicitly required by the DWD, they offer significant value within this RBA as a safety net to detect new or re-emerging pollutants and assess biologically active – and thus possibly harmful – compounds. Dutch drinking water companies apply bioassays in this context, benchmarking responses against effect-based trigger values (EBTs). When EBTs are exceeded, effect-directed analysis (EDA) identifies causative compounds, enabling targeted risk management.

In September 2025, the European Commission announced EBMs will be incorporated into the WFD for surface waters, with mandatory monitoring of estrogenic activity for two years. Additionally, EBMs can be employed within the WFD to detect mixture effects and unknown pollutants that may hinder attainment of a 'good ecological status'. Therefore, a Dutch research consortium developed and applied the Key Factor Toxicity (KFTox) instrument, combining chemical and bioassay tracks to assess toxic pressure in water and its potential ecological impact.

These examples demonstrate that applying EBMs within regulatory water quality assessment is possible. Nevertheless, EU-wide implementation faces challenges, including standardization, cost, and consensus on EBTs. To address these, we initiated a European survey targeting stakeholders and experts in water management, research, and regulation. The survey aims to identify barriers and opportunities for EBM adoption, evaluate knowledge gaps, and explore strategies for harmonization. Outcomes will provide insights to guide policy development, supporting broader integration of EBMs into regulatory frameworks.

1.13 Integrating Chemical Pollution and Wastewater-Based Epidemiology into the One Health Framework

1.13.T-01 HydroFATE as a One Health Tool: Modelling Multi-Contaminant Risks from Different Sources and Across Scales

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Chemical pollution is a critical yet underexplored driver of health outcomes across the human–animal–environment interface, and comprehensive monitoring is often constrained by resource and analytical limitations. To address this gap, we present new applications of HydroFATE, a global contaminant fate model designed as an accessible screening tool for assessing chemical risks in surface waters. HydroFATE integrates spatially explicit pollution sources, hydrological connectivity, and chemical-specific parameters to enable rapid, scientifically robust assessments for stakeholders, including regulators, industry, and water managers. Within a One Health framework, we apply HydroFATE to case studies in collaboration with international partners, including modelling veterinary and domestic antibiotic pollution in Canadian waters and assessing the environmental risk of 80 chemicals representative of typical wastewater composition in European rivers. Despite the added complexity of these applications, HydroFATE retains its core strength as a streamlined platform for screening and prioritization. Initial results reveal overlapping contamination patterns that may exacerbate antimicrobial resistance and ecological stress, underscoring the importance of multi-contaminant approaches to risk assessment. Although development of a user-friendly interface is underway, the current framework already supports evidence-based policy development and proactive strategies for safeguarding water quality and ecosystem health. Ongoing efforts to expand HydroFATE's contaminant library and foster collaboration with government agencies, non-profit organizations, and industry stakeholders will further enhance its utility for integrated water management worldwide, ultimately contributing to the preservation of global water quality and the protection of interconnected human, animal, and environmental health.

1.13.T-02 Proactive public health – actionable data for notifiable diseases using wastewater-based epidemiology

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Having been used to estimate lead times for SARS-CoV-2 infection surges, thereby facilitating early interventions to reduce spread, wastewater-based epidemiology and surveillance (WBE/WBS) has subsequently been used for many additional pathogens. Although reverse transcription-quantitative polymerase chain reaction (RT-qPCR), RT digital PCR (RT-dPCR) and RT digital droplet PCR (RT-ddPCR) are commonly used for WBE, differences among the platforms exist.

In this study, we employed RT-qPCR and RT-dPCR platforms for the comparative detection of vaccine (A genotype Edmonston) and wild-type (D8 genotype) measles RNA from wastewater in nearby cities separated by < 50 kilometers during the 2025 measles outbreak in Texas, USA, in addition to several other locations up to 30 – 1,400 kilometers from the outbreak location. In addition, as measles is also a mandatory notifiable disease, clinical testing for measles is required if patient present with symptoms consistent with measles. Each wastewater sample with a positive of D8 using PCR (n = 23), was also sequenced, with 22 samples being successfully sequenced. Of the successfully sequenced samples, 13 were used for phylogenetic tree reconstruction. Each of these 13 wastewater samples could be directly matched to a clinical genome, with seven wastewater samples having zero SNP differences to clinical cases, four wastewater samples having +1 SNP, and one showed -1 SNP. Of these 13 samples, three (> 20 %) provided early warning. Many of the influent samples led clinical genome confirmation by 9 – 19 days (i.e. detection in wastewater prior to clinical detection), although not all, with prolonged shedding also observed.

This study, a first to specifically connect individual clinical samples with population level wastewater surveillance, demonstrates a widening use case for WBE, providing actionable data for notifiable diseases, capturing transmission dynamics that extend beyond geographic boundaries.

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1.13.T-03 A National-Scale Application of Wastewater Analysis to Monitor Plasticizer Exposure in Spain

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The widespread use of plastics in the 20th century led to the incorporation of additives such as BPA and phthalates, later identified as endocrine disruptors. Their regulation prompted the introduction of alternative compounds (bisphenol S or F, terephthalates, di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH)), whose toxicological properties remain insufficiently understood, highlighting the need for robust monitoring of both traditional and emerging plastic-related contaminants. Wastewater-based epidemiology (WBE) offers an efficient tool to assess population exposure through its metabolic biomarkers in wastewater. Although these compounds have been studied in several countries, data from Spain are limited and lack national coverage. Hence, this study investigates human exposure to 3 bisphenols and 11 plasticizers (from phthalate, terephthalate and DINCH families) by measuring their metabolites in influent wastewater collected in 2023 from 27 WWTPs across Spain, representing over six million inhabitants. Samples underwent solid-phase extraction and were analyzed by liquid chromatography coupled to tandem mass spectrometry. Then, concentration levels were transformed into population-normalized daily loads (PNDLs) applying the flow rate and the population served by the WWTP. Findings show that short-chain phthalates remain prevalent, with DEP indicating the highest overall exposure (~500 mg/day/1000 inhabitants) whereas in a northern region, DEHP dominated, exceeding 2000 mg/day/1000 inhabitants. Among phthalate alternatives, DEHTP reached levels comparable to its phthalate analogue, while DINCH appeared sporadically at < 100 mg/day/1000 inhabitants. For bisphenols, metabolites of BPA and BPS were detected in all samples, whereas BPF appeared in fewer than half of the cities. Generally, BPS exposure exceeded BPA (~200 vs. ~100 mg/day/1000 inhabitants), except for one northwestern city showing unusually high BPA levels.

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1.13.T-04 Bridging Environment to Exposome: In Vitro Evidence for Chemical Mixture Propagation to Humans

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Synthetic organic chemicals are pervasive in the environment, forming complex mixtures with diverse toxic potentials. Their number, diversity, and co-occurrence pose major challenges for assessing risks to ecosystems and human health, particularly at low concentrations. In vitro bioassays are well-suited for evaluating both known and unknown mixtures. In the Panoramix project [1], we examined how environmental chemicals

propagate through food webs to humans. Samples from 5–10 European countries were pooled, covering wastewater influent and effluent (human waste and emissions), surface water and fish (aquatic and dietary exposure), and conventional and organic cow milk (terrestrial mammal exposure). Human dietary exposure was represented by drinking water, bottled water, milk, and fish, while breast milk, adult serum, and cord serum captured direct human exposure. All samples were extracted using harmonized protocols and analyzed with targeted and nontarget chemical screening [2]. Extracts and reconstituted mixtures of 24 commonly detected chemicals were tested in 22 in vitro bioassays covering neurotoxicity, thyroid disruption, reproductive toxicity, and genotoxicity. Most bioassays responded, with strongest effects in samples from higher trophic levels like fish and humans. Reconstituted mixtures conformed to concentration addition predictions, but accounted for only a fraction of the observed bioactivity—pointing to the presence of uncharacterized toxicants and underscoring the limitations of current analytical coverage. These findings illustrate how synthetic chemicals cross ecological and physiological boundaries, impacting ecosystems and human health alike, highlighting the value of in vitro tools and a One Health approach to integrated risk assessment. [1] Escher et al. (2022) *Int J Environ Res Public Health*, 19, 12990. [2] Motteau, et al. (2025). *Environ Sci Technol*. 10.1021/acs.est.4c12608. PANORAMIX project <https://panoramix-h2020.eu/>.

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1.13.T-05 Potential of Settling Basins to Record Quantitative Information about Public Health: History of Paris since 2018

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Settling basins in sewage networks mainly trap coarse particles that accumulate between cleanings. These deposits, though coarse, can archive contamination signals from wastewater. With the rapid development of wastewater-based epidemiology (WBE), which estimates community health and consumption from wastewater composition, this study explores whether sediments in an urban settling basin can serve as historical archives of both chemical and microbiological anthropogenic contamination.

We investigated the Marceau–Courcelles settling basin (northwestern Paris), a cylindrical underground structure receiving mixed wastewater and stormwater, not dredged since 2018. In 2024, four sediment cores (0.5–1 m) were collected and subsampled (48 samples) for grain size, total organic carbon (TOC), organic matter characterization (Rock-Eval), chemical contaminants (LC–MS/MS after ASE extraction), and microbial indicators quantified by digital (RT)-PCR (CrAssphage, *E. coli*, PMMoV, adenoviruses, noroviruses). Sediment stratigraphy and monitoring data allowed correlation between depth and time, reconstructing contamination trends from 2018–2024, including the COVID-19 period.

Twelve depositional layers (mostly winter) were identified, with lateral heterogeneity in sedimentation but similar geochemistry across cores. Sand dominated (70–90%), though TOC reached up to 9.5%, without clear correlation to grain size. Among 60 targeted molecules, 42 were detected (up to several $\mu\text{g g}^{-1}$), showing both vertical and lateral variability independent of in situ degradation. Statistical associations between parent compounds and metabolites (e.g., cocaine/benzoyllecgonine, caffeine/paraxanthine) suggest variations in wastewater inputs rather than diagenetic processes.

Microbiological markers showed variable behavior: *E. coli* and CrAssphage were irregular, whereas PMMoV, adenoviruses, and noroviruses displayed consistent patterns and NVG I/II ratios correlated with wastewater signals. SARS-CoV-2 was not detected, likely due to degradation.

Overall, sediments captured temporal variability in chemical and microbial markers, confirming their potential as retrospective WBE archives. The Marceau–Courcelles basin thus potentially functions as an urban observatory, archiving contamination and public health signals. These findings open perspectives for using urban sediment records to reconstruct past pollution and epidemiological trends, complementing real-time wastewater monitoring.

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1.13.P Integrating Chemical Pollution and Wastewater-Based Epidemiology into the One Health Framework

1.13.P-We060 From Point Measurements to Integrated Assessments: How Decision Theory Supports Integration Over Multiple Objectives, Space, and Time

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Environmental assessments involve diverse objectives, temporal and spatial scales, making integration a major challenge. Multi-criteria decision analysis (MCDA), rooted in decision theory, provides a structured approach to address this complexity: By disaggregating overarching objectives into smaller components that can be assessed separately, it provides a framework to make wicked problems manageable. By aggregating the smaller components into holistic assessments, it facilitates synthesis. Its foundation in decision theory ensures that assessment procedures satisfy mathematical axioms essential for rational decision making.

As a framework to formulate, harmonize and integrate assessments, MCDA offers tools for elicitation, implementation and visualization. This facilitates information to be presented at different levels of detail, supporting a transparent communication tailored to specific management questions and audiences.

A key challenge for integration is selecting appropriate mathematical aggregation methods. This contribution examines their implications and provides practical guidance for choosing methods suited to specific contexts to prevent unintended consequences through informed methodological choices.

We illustrate applications of MCDA for integrated assessments in the scope of surface waters and at different scales. Examples include the integration over multiple pollutants and ecotoxicological endpoints for integrated water quality assessment, the integration of physical, chemical and biological modules into an ecological assessment of rivers at the reach scale, or the integration of spatial criteria for the prioritization of the removal of fish migration barriers at the catchment scale.

With the potential of MCDA to harmonize and integrate diverse data sources and indicators, it can support advancing One Health-oriented risk assessment and management frameworks.

1.13.P-We061 Impact of Widely Occurring Organic Micropollutants on Antibiotic Resistance in Clinically Relevant Bacterial Pathogens

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Antibiotics are widely used antimicrobial agents, but their extensive application exerts strong selective pressure on pathogens, contributing to the global rise of antibiotic resistance. Recent studies show that not only antibiotics but also non-antibiotic anthropogenic micropollutants can promote the development and spread of resistance genes. Given their global application and frequent environmental detection, we selected three pesticide active substances and three personal care product ingredients to evaluate their potential to induce resistance in environmental isolates of clinically relevant opportunistic pathogens.

The examined strains (*Escherichia coli*, *Acinetobacter johnsonii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*) belonged to ESKAPEE-associated genera.

MIC experiments were preceded by cytotoxicity tests to determine sublethal micropollutant concentrations (<EC₁₀). Bacterial cultures were then exposed for 96 h to 10 mg/L micropollutants, followed by standardized MIC testing (EUCAST/CLSI). Resistance profiles of untreated “wild-type” strains served as references.

Our results show that the tested micropollutants frequently induced increased MIC values across all species. *E. coli* displayed 2–3-fold increases against ciprofloxacin; imipenem resistance emerged after exposure to benzophenone-3, glyphosate, and DEET. In *A. johnsonii*, all compounds induced ciprofloxacin resistance, and several caused up to fourfold MIC increases for piperacillin–tazobactam. In *K. pneumoniae*, all micropollutants triggered ciprofloxacin resistance, with glyphosate, benzophenone-3, and methylparaben showing the strongest effects. *P. aeruginosa* showed the most alarming pattern: nearly all micropollutants induced resistance to almost all tested antibiotics. In *S. maltophilia*, shifts in MIC values suggested altered susceptibility for multiple drug classes, including carbapenems and fluoroquinolones.

Our findings demonstrate that environmentally relevant micropollutants—though not antibiotics—can substantially contribute to the development of antibiotic resistance in facultative pathogenic environmental bacteria. These results highlight the critical role that non-antibiotic organic micropollutants may play in shaping resistance patterns in environmental bacteria, reinforcing their relevance within the One Health perspective.

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1.13.P-We062 The AMR Multi-Stakeholder Partnership Platform: a global Quadripartite initiative to address the challenges of antimicrobial resistance by fostering collaboration between diverse stakeholders

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Antimicrobial resistance (AMR) is a global health threat arising from the misuse of antimicrobials, leading to infections becoming harder to treat in humans, animals and contaminating the environment. This resistance threatens modern medicine, food security, social and economic stability, making common infections life-threatening and compromise medical procedures. As it spans borders and sectors, addressing AMR requires a collaborative, One Health approach involving all stakeholders to address the interconnectedness of humans, animals, plants and the environment. Despite decades of effort, governance remains fragmented, scientific knowledge is unevenly translated into policy, and inequities in access to antimicrobials fuel inappropriate use. In response, the Quadripartite organizations (FAO, UNEP, WHO and WOA) established in 2022 the AMR Multi-Stakeholder Partnership Platform (MSPP) to mobilize collective action, integrate underrepresented perspectives, and accelerate implementation of evidence-based solutions applying the One Health approach. The MSPP engages more than 300 entities, organized in 5 clusters and 13 Action Groups which are operational units, testing of practical interventions.

The MSPP elevates underrepresented stakeholder groups and sectors into global AMR decision-making processes, facilitating widespread stakeholder participation in the 2024 UN General Assembly High-Level Meeting on AMR and the Fourth High-Level Ministerial Conference in Jeddah. In 2025, it organized global consultations for the update of the Global Action Plan on AMR and the establishment of the Independent Panel on Evidence for Action against AMR (IPEA), enabling contributions from over 2,000 participants across sectors and regions. This approach exemplifies a multi-stakeholder governance model in which heterogeneity of expertise is treated as a strategic asset rather than a barrier to problem-solving.

The MSPP, in an era of declining trust in multilateral institutions, financial constraints and policy fragmentation, provides a structured mechanism for integrating scientific evidence, diverse experience and collective priority-setting into coordinated global action. By strengthening connectivity across sectors and disciplines, it contributes to a more resilient, equitable and effective response to one of the most pressing public health threats of the 21st century.

1.13.P-We063 Ecotoxicity of selected antimicrobial resistance (AMR) causing agents in potentially susceptible species

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While antimicrobial resistance (AMR) caused by the use of antibiotics in patients and livestock has attracted considerable attention, the ecotoxicological risks of AMR-causing agents remain a significant concern. In Japan, some macrolide antibiotics are considered among the highest-risk pharmaceuticals—second only to hormonal drugs—based on screening-level risk assessments. However, there are still substantial ecotoxicity data gaps for this group of chemicals, as toxicity testing with the standard three species (green alga, daphnid, and fish) is not required for human or veterinary medicines under current Japanese regulations.

Since 2002, we have conducted screening-level risk assessments for over 400 chemicals under the “Initial Risk Assessment of Chemicals” program of the Ministry of the Environment, Japan. Yet, only eight of these chemicals have been antimicrobials (e.g., clarithromycin, sulfamethoxazole). To address data gaps, we performed ecotoxicity tests not only with the standard three species but also with potentially susceptible organisms such as cyanobacteria, luminescent bacteria, and protozoa (*Tetrahymena* sp.). The results of these studies were presented at the 46th Annual Meeting of SETAC North America in Portland, OR, USA.

We subsequently shifted our focus to other antimicrobials considered potential contributors to AMR. For these substances, we conducted ecotoxicity tests using both the standard three species (green alga, daphnid, and fish) and the additional susceptible species (cyanobacteria, luminescent bacteria, and protozoa). Based on the newly

obtained ecotoxicity data and existing monitoring data, we also carried out screening-level risk assessments for these antimicrobials.

This research was supported by the Environment Research and Technology Development Fund (JPMEERF25S21230) of the Environmental Restoration and Conservation Agency (ERCA), provided by the Ministry of the Environment of Japan.

1.13.P-We064 Prevalence of Selected Antibiotic Resistance Genes in Agricultural Soils in Germany

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Growing concerns about the presence of antibiotic resistance genes (ARGs) in agricultural soils—especially due to inputs such as manure—have highlighted the need to better understand environmental reservoirs of antimicrobial resistance and their potential risk to human and animal health. In this context, soils from 63 agricultural sites across Germany were sampled between 2022 and 2024 and analyzed for a wide range of parameters, including soil physicochemical properties, contamination with plant protection products and pharmaceuticals, ecological variables, and the presence of ARGs. In addition to the agricultural sites, three reference sites retrieved from the German Environmental Specimen Bank were included in the ARG analysis to provide background levels for comparison. This integrated dataset allowed the assessment of potential links between ARG abundance and agricultural practices, chemical exposure, and soil characteristics across Germany. The study showed that a few ARGs were widespread across German agricultural soils. Genes conferring resistance to macrolide (ermB) and tetracycline (tetA) classes were most frequently detected. In contrast, genes associated with aminoglycoside resistance (aacC4 and aadA1) and trimethoprim resistance (dfrA1) were only sporadically detected. Genes from additional classes—including macrolides (ermA), trimethoprim (dfrA12), β -lactams (blaCTX-M1), quinolones/fluoroquinolones (qnrS), and polypeptide antibiotics such as colistin (mcr-3)—were not detected, indicating their low abundance or absence in analyzed soil samples. The class 1 integrase gene (intI1), as an indicator of mobile genetic elements, was present at nearly all sampled sites. Relative ARG abundances varied between sites and tended to be higher in soils with manure or digestate application. In contrast, reference sites—representing soils with minimal or no anthropogenic influence—generally exhibited lower ARG prevalence, suggesting that agricultural activities and organic fertilization contribute to the enrichment and spread of resistance genes in soil environments. Although direct causal relationships between antibiotic residues and ARG occurrence could not be established, the observed co-occurrence highlights the importance of evaluating together these factors when assessing the spread of antibiotic resistance in the environment.

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1.13.P-We065 In Vivo Toxicity and Ecotoxicological Evaluation of a Bioactive Metabolite Derived from *Streptomyces nogalater* KNB14980

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Microbial-derived crop protection agents require comprehensive evaluation of their in vivo toxicity and ecological safety to ensure reliable field application. In this study, we investigated the in vivo and ecotoxicological properties of a bioactive metabolite (KNB14980 EM, 98% a.i.) produced by *Streptomyces nogalater* KNB14980 following standardized testing protocols.

Acute oral and dermal toxicity assessments, along with skin and eye irritation tests, were conducted in rodent and rabbit models. Oral administration of up to 2000 mg/kg in female Sprague–Dawley rats resulted in no mortality or treatment-related adverse effects. Dermal exposure at 4000 mg/kg induced only transient and fully reversible skin reactions. Skin and eye irritation tests in rabbits showed no evidence of corrosion or persistent damage, confirming the compound to be non-irritating.

Ecotoxicity was further examined through acute aquatic and pollinator assays. Danio rerio exhibited no abnormal responses, with 48-h and 96-h LC50 values exceeding 100 mg/L. Honeybee (*Apis mellifera*) oral and

contact exposure both yielded LD50 values greater than 100 µg a.i./bee, indicating low risk to pollinators. Collectively, these findings demonstrate that the metabolite possesses low acute in vivo toxicity, negligible irritation potential, and minimal hazard to aquatic organisms and pollinators. The results support its suitability as a safe and environmentally compatible microbial-based crop protection agent.

Disclaimer/Disclosure:

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1.13.P-We066 Determining Potentially Misused Pharmaceuticals and Metabolites to Assess Usage Through Wastewater Analysis

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Wastewater-based epidemiology (WBE) is a relatively new approach that provides insight into pharmaceutical consumption at the population level. Traditional methods rely on sales and prescription data, which can yield inaccurate results, especially for pharmaceuticals that are frequently misused. WBE can therefore serve as a complementary method to provide more accurate consumption figures.

The aim of this research is to develop and validate an analytical method for the simultaneous determination of 39 pharmaceuticals and their metabolites in wastewater, including anxiolytics, hypnotics, and phosphodiesterase type V inhibitors. The analysis will be conducted using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), with solid-phase extraction (SPE) enrichment.

After optimizing mass spectrometric parameters and chromatographic separation, the SPE procedure was developed and optimized. The target compounds were enriched from 10 mL raw wastewater samples using hydrophilic-lipophilic balance cartridges, with an average recovery of 90%. Method accuracy was also high (average trueness: 90%; average precision: 5%), and low detection limits were achieved. Enzymatic deconjugation experiments were conducted to assess the contribution of the conjugated forms of selected compounds, particularly benzodiazepines, while in-sample model experiments were performed to evaluate the stability of the target compounds. Most compounds were highly stable at both 4 °C and 25 °C over 24 hours, indicating negligible transformation during composite sample collection and short-term storage, confirming their suitability for WBE.

The developed method will be used to assess spatial and temporal trends in the consumption of selected pharmaceuticals in six Croatian regions using the WBE approach. These results will be compared with data on pharmaceutical consumption obtained through traditional methods to assess potential misuse.

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1.13.P-We067 Informing Site Selection for Wastewater-Based Epidemiology with Social Vulnerability Indicators

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Though wastewater-based epidemiology (WBE) can be used to understand socioeconomic status, it may also be possible to identify disease susceptibility. We recently sampled the wastewater treatment plants (WWTPs) of two communities of similar size that vary notably in their socioeconomic status, with one community ranked among the healthiest in the state of Texas, USA, and having low social vulnerability, while the other community is ranked as moderately healthy with medium-high social vulnerability. When these samples were analyzed for 24 pharmaceuticals, wastewater tracers, and an illicit drug metabolite, we observed significantly ($p < 0.001$) higher consumption of pharmaceuticals and an illicit drug at the site with higher social vulnerability. Similarly, a growing body of work considers the influence of socioeconomic factors on the loading of pharmaceuticals to the environment from WWTP effluent discharges. To better understand this relationship across a gradient of urbanization and socioeconomic status, we selected 14 estuarine sites across the Gulf Coast of Texas, USA, collecting paired time-weighted composite and grab samples across two seasons. In both seasons, we identified a significant ($p < 0.05$) negative relationship between sum pharmaceutical mass load and county health ranking,

suggesting greater environmental loading of pharmaceuticals in healthier communities with low social vulnerability. This approach also shows the potential use of WBE methods to identify hotspots with high chemical loads and thereby inform environmental monitoring, assessment and management. A key issue that limits the application of much socioeconomic data to WBE is the relatively limited spatial resolution of data; for example, in the US, much data is reported only at the county or zip code level, limiting the usefulness of this data to derive insights about sewersheds that often contain only portions of counties or zip codes. To begin to close this data gap, we developed a sewershed-level social vulnerability index across all United States WWTPs; our ongoing studies are examining the utility of this approach by analyzing illicit drugs, wastewater tracers, and viral targets in several Texas WWTPs located across a gradient of socioeconomic conditions. This new sewershed social vulnerability index, which is based on census tract information (~4,000 people), presents an opportunity to spatially inform future environmental monitoring, assessment and management efforts.

1.13.P-We068 Assessment of population exposure to polycyclic aromatic hydrocarbons using wastewater-based epidemiological analysis

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Wastewater-based epidemiology (WBE) may be able to monitor public health emergencies by analyzing human urinary biomarkers in wastewater. This work proposes a novel approach utilizing WBE for the spatial and temporal evaluation of PAHs exposure using hydroxyl derivatives of PAHs. These are 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 9-hydroxyfluorene, 9-hydroxyphenanthrene, 1-hydroxypyrene and 3-hydroxybenzo(a)pyrene. Most target markers were found at quantifiable concentrations in raw and treated wastewater. The total loads identified in raw sewage ranged from 88.33 g/day to 154.77 g/day during the summer period and from 137.66 g/day to 283.78 g/day during the winter period. The obtained results for the removal efficiencies of OH-PAHs indicate a seasonal dependency in their degradation. Removal efficiencies were higher in January compared to August. The results of the back calculations allowed to estimate that during the summer, on average, a resident of Krakow could absorb approximately 2.1 µg of the assessed OH-PAHs per day, while in winter, this value increased to 4.1 µg. This is close to the reported in the literature value that the total daily exposure to OH-PAHs is estimated at 3 µg/day. To the authors' knowledge, this is the first time wastewater profiling of OH-PAHs in wastewater for the evaluation of exposure to PAHs have been used, also their removal as well emission with effluent were determined.

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1.13.P-We069 Unveiling the presence of 20 perfluoroalkyl substances in wastewater in Spain – a national inventory of household emissions

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Per- and polyfluoroalkyl substances (PFAS) and specially perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) have been extensively manufactured for numerous industrial and consumer applications. Several studies have suggested that both acute and chronic health effects, including cardiovascular diseases, may be associated with PFAS exposure, prompting the European Commission to impose restrictions on their use. Hence, the occurrence and fate of PFAS in wastewater and during treatment has been a hot topic in the last years. Nonetheless, few studies have considered this problematic in Spain, while there are no investigations at national level, nor have put the focus on using wastewater data to assess emissions.

In this context, this study aims to provide a comprehensive assessment of the occurrence of 20 PFAS across Spain by analyzing influent wastewater samples from 27 wastewater treatment plants (WWTPs) collected in 2023, covering a population of over six million inhabitants. Samples were solid-phase extracted, injected in a liquid chromatography-tandem mass spectrometry system and PFAS concentrations were transformed into population-normalized daily loads (PNDLs) applying the flow rate and the population served by the WWTP. Eleven PFAS were positively detected in influent wastewater samples being, 7 PFCAs (from C4 to C10) and 4 PFSAAs (C4, C6, C8 and C9). The highest detection frequency was denoted for the PFCAs family, highlighting perfluorobutanoic, perfluoroheptanoic and perfluorooctanoic acid that were detected in all wastewater samples. Similar PNDLs were found for detected PFAS with perfluorobutanoic acid showing the highest PNDLs (median: 8.9 mg/day/1000inhabitants) followed by the two C6 PFAS (median of 3.8 mg/day/1000inhabitants and 3.2 mg/day/1000inhabitants for perfluorohexanoic acid and perfluorohexanesulfonic acid, respectively). Regional patterns showed minimal differences overall, except for four PFCAs, for which exposure levels were found to be twice as high in a central region of Spain (median: 21 mg/day/1000inhabitants). Hence, the national extrapolation of the total PFAS-related PNDLs daily discharged to Spanish WWTPs is estimated at 1.7 mg per inhabitant per day.

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1.13.P-We070 UHPLC–MS/MS Analysis of Lifestyle and Dietary Biomarkers in Wastewater: Method Optimization, Biomarker Stability, and Multi-city Study

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Wastewater-based epidemiology (WBE) has become a powerful tool for assessing population exposure to illicit drugs and other lifestyle chemicals. However, its potential for evaluating dietary habits remains largely unexplored due to the vast number of food-related biomarkers. To address this, we developed a UHPLC–MS/MS method to quantify over 40 lifestyle and dietary biomarkers in wastewater. These analytes cover diverse classes: flavonoids (plant metabolites) and other food constituents, vitamins, artificial sweeteners, caffeine, nicotine and alcohol biomarkers, and the pharmaceutical metformin, including many not previously monitored in WBE.

A dual sample preparation workflow was optimized to accommodate the wide range of analyte concentrations: high-abundance compounds (e.g., caffeine, nicotine and alcohol metabolites, sweeteners, metformin) were quantified by direct injection of diluted wastewater, whereas low-abundance dietary biomarkers were enriched using solid-phase extraction on hydrophilic-lipophilic balance cartridges. All compounds were separated on a T3 HSS C18 column using an acidified water–acetonitrile gradient and detected by tandem mass spectrometry in multiple reaction monitoring mode. The method achieved excellent linearity ($r^2 \geq 0.99$), high recoveries (>80% for most analytes), and quantification limits low enough to ensure reliable determination.

Stability tests showed that most biomarkers remained stable at 4 °C for 24 h, whereas some, such as certain vitamins and polyphenols, degraded significantly during prolonged storage at –20 °C. These findings highlight the need for prompt analysis or consideration of analyte losses. The method was applied during the 2025 one-week autumn/winter sampling campaign, analyzing 24-h composite wastewater samples from 12 Croatian cities across six regions, and revealed spatial variations in lifestyle and dietary habits.

Our findings broaden the scope of WBE by enabling high-throughput monitoring of dietary biomarkers alongside traditional surveillance of substances of abuse and other lifestyle chemicals. Future work will assess the in-sewer stability of biomarkers to validate their robustness under sewer conditions, and an additional sampling campaign in spring will investigate seasonal differences in lifestyle and dietary habits.

Disclaimer/Disclosure:

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1.13.P-We071 A temporal analysis of microbiome dynamics in activated sewage sludge aerated for 14 days in OECD 301 biodegradation tests

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Organisation for Economic Co-operation and Development (OECD) 301 guidelines provide the basic requirement for microbial counts from activated sewage sludge for each of the six tests under this guideline. The guideline also states that the activated sewage sludge may be kept aerated for up to seven days. However, the microbiome of activated sewage sludge is highly dependent on the domestic population from which it is derived, with several genera of bacteria present in greater abundance over a myriad of bacterial, fungal, viral, and protozoal genera. Such diverse microbial populations provide enzymatic and metabolic pathways for biodegradation studies, such as oxygenases, hydrolases, and dehydrogenases. As microbial diversity changes across the duration of activated sewage sludge aeration, studies have shown reductions in enzymatic expression. Therefore, the aim of the present study is to determine the change in microbiome profile, with an emphasis on bacterial communities, over a maximum of 14 days aeration, with colony forming unit (CFU) counts associated with respective OECD 301 tests throughout the 14 days. Metagenomic methodologies were employed to determine microbiome diversity, showing a significant alteration ($P < 0.001$) in bacterial diversity. Primary biodegradation species, such as *Pseudomonas fluorescens*, significantly increased ($P < 0.001$) in abundance up to day seven with greatest abundance on day four. Aerobic CFU counts indicated a consistent decline between days two and eight ($P < 0.001$), corresponding to the metagenomic diversity changes across the same time period. Post day eight, CFU counts increased to day 14, however, metagenomic data indicated such an increase of CFU counts was a migration away from typical biodegradation species. Therefore, based on both cell culture and metagenomic analysis, the present study is able to conclude that activated sewage sludge for OECD 301 tests would be more appropriately kept under aeration for a total of five days as opposed to the typical seven days in order to utilise the select key biodegradation bacterial species

1.13.P-We073 The Use of in vitro Bioassays To Assess the Endocrine Disrupting Potential of 4-tert-octylphenol via Estrogen Receptors Vital to the Regulation of Female Fertility.

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Alkylphenols (APs) are a family of compounds used in a wide range of commercial and industrial products. APs are commonly used to synthesise alkylphenol ethoxylate surfactants, which degrade in the environment back to APs such as 4-tert-octylphenol (4-t-OP). With no current widespread wastewater removal methods preventing its entry to the ecosystem, 4-t-OP is continually detected across many environmental matrices. Continual exposure to 4-t-OP is of concern due to its well documented estrogenic activity and association with reproductive toxicity. However, studies have mainly focused on reproductive effects in wildlife and in males, while the exact effects of 4-t-OP on female reproductive processes remain less understood, particularly regarding its ability to disrupt estrogen receptor (ER) activity which is vital to the regulation of fertility. Previous research has shown that 4-t-OP exposure triggers ER transactivation by utilising Reporter Gene Assay (RGA) cell lines expressing ER α and ER β respectively, but these results alone cannot convey how endogenous ER is affected in vivo, since both isoforms are usually co-expressed. To address this, we assessed the effect of 4-t-OP exposure on ER transactivation by measuring luciferase response using an RGA cell line (MMV-Luc) expressing both ER α and ER β , which mimics the in vivo scenario whereby these receptors communicate. Sample toxicity was monitored in the RGA using the MTT assay. The effect of 4-t-OP exposure on ER translocation was also measured by using U2OS cells stably expressing fluorescently tagged ER α and ER β respectively and imaged using High Content Analysis. Sample toxicity was controlled by monitoring pre-lethal cytotoxic effects in the same assay.

An increase in both ER translocation and transactivation in the presence of human relevant exposure levels of 4-t-OP was observed, indicating ER agonism, and extension beyond these levels revealed dose-response relationships, with translocation results showing a higher response for ER β .

In summary, 4-t-OP can disrupt ER receptor signalling. The correlation of results between both assays also validates the newer generation translocation assays which provide additional data output including sample toxicity control. These findings suggest that exposure may negatively impact female reproductive processes, highlighting the need to continue toxicological screening of commercially relevant compounds detected in the environment.

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1.13.P-We074 One Health Insights Into Malathion Toxicity: Protective Effects of Thymoquinone and Diallyl Sulphide in Rats

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Malathion, a widely used organophosphate insecticide, poses serious human and animal health concerns due to its ability to inhibit acetylcholinesterase and disrupt multiple biochemical and oxidative pathways. Understanding how natural bioactive compounds can mitigate pesticide-induced toxicity is essential within a One Health framework, where chemical exposures affect humans, animals, and shared environments.

This study aimed to evaluate the protective effects of thymoquinone (TQ) and diallyl sulphide (DAS) against malathion-induced oxidative and biochemical disturbances in rat cerebral, hepatic, and renal tissues. To address this challenge, rats were exposed to malathion with or without co-treatment using TQ or DAS for 30 days. Blood and tissue samples were subsequently collected to assess liver and kidney biomarkers, antioxidant enzyme activities, and oxidative stress indicators.

Malathion administration resulted in significant increases in serum AST, ALT, ALP, LDH, GGT, cholesterol, urea, creatinine, and 8-OHdG, along with reductions in total protein, albumin, triglycerides, and AChE. In parallel, malathion markedly elevated tissue malondialdehyde and nitric oxide levels while suppressing glutathione and antioxidant enzymes (GPx, SOD, CAT). Co-treatment with TQ or DAS significantly ameliorated these alterations, with TQ showing the most consistent restoration toward normal physiological ranges.

In conclusion, both TQ and DAS effectively mitigated malathion-induced biochemical and oxidative damage, likely by reducing reactive oxygen and nitrogen species and enhancing endogenous antioxidant defenses. These findings highlight the potential of natural protective agents in reducing pesticide-related health risks and underscore the relevance of integrating animal toxicity data into broader human and environmental health considerations within a One Health perspective.

1.13.P-We075 (Eco)toxicological effects of nanoengineered pesticides on humans and non-target biota

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Nanoengineered pesticides are novel chemical products that target pests with sufficient efficacy to maintain plant health. However, our knowledge regarding their environmental fate and exposure is still insufficient and we cannot reliably assess for instance potential accumulation across several biological organizations, and the potential ecotoxicological impacts. Several studies have already pointed out specific impacts at different scales of biological organization, but still it is not clear if and how this information could be utilized in a regulatory risk assessment framework. A systematic literature review was conducted to assess: (i) the advances in nanomaterial application in relation to classical pesticide use; (ii) if and which of the impacts on the organisms of the environment from nanomaterials used in pesticides, are assessed or sufficiently investigated to serve for regulatory risk or effect assessment schemes; (iii) if we should really focus on specific toxicological impacts following nano-based pesticide applications on the environment, that are supposed to have a limited exposure and thus limited effect on organisms; (iv) if and which of the impacts on organisms from nanomaterials used in pesticides, are assessed or sufficiently investigated to serve for regulatory risk or effect assessment schemes; and (v) the type of effects that have so far been detected in the available literature. Articles describing results of experimental research on toxic effects from nano-based pesticides on non-target biota were screened and gathered using the ASReview product developed by Utrecht University, and PRISMA guidelines on reporting towards meta-analyses. Impacts-related data are classified and analyzed. This review will enhance: (i) our understanding of the gaps that still need to be covered in the field of ecotoxicology for nano-pesticides, and (ii) our reporting of the available (eco)toxicological data from studies with organisms, for nano-enabled pesticides used in the agrochemical sector for plant protection. This advance would potentially minimize adverse effects on environmental and human health.

1.13.P-We076 Impact of E-cigarettes on Invertebrates in Aquatic Ecosystems

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E-cigarettes, especially disposable e-cigarettes, have been less researched than traditional cigarettes. In addition to alkaloids, they contain heavy metals and complex chemical mixtures.

A student experiment has shown that not only does the mechanical destruction of the cigarettes pose a fire hazard, but also that significant amounts of the solutions remain in the sponge contained within them. Furthermore, the toxic effect appears to occur much more quickly than with normal cigarettes, as there is no release from the tobacco, but the harmful substances are already present in liquid form. This leads to significantly faster bioavailability. We present initial results from experiments conducted in cooperation with schoolchildren showing that e-cigarettes pose an environmental risk on invertebrates.

1.13.P-We077 Heavy metals analysis in selected wetlands in the Vaal Region, South Africa: A shift towards achieving Sustainable Development Goals

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Wetlands offer wide array of ecological, economic, and social benefits. However, these freshwater environments are gradually degraded and lost through anthropogenic activities, climate change and pollutants, including heavy metals globally. Heavy metals are toxic, persistent, and non-degradable contaminants that enter wetlands through human activities, threatening both aquatic organisms and humans. The present study aimed to analyse heavy metals in selected wetlands in the Vaal Region, South Africa. Using Nanocolor test kits with the 500D photometer, heavy metals were analysed from water samples collected at predetermined points, at the input and output along the wetlands during dry and wet seasons. Findings were compared with wastewater limit values applicable to the discharge of wastewater into water resources. Results for dry season (input: output) were as follows: Copper: 0.81; 0.72 mg/L, Lead (1.16; 0.84 mg/L, Silver (0.50; 0.30) and Zinc (0.50; 0.24). For wet season: Copper (0.46; 0.27mg/L), Lead (1.01; 0.89 mg/L), Silver (0.59; 0.25mg/L) and Zinc (0.48; 0.28mg/L). Low heavy metal concentrations at the outputs in both seasons in all sampled sites were indicative of reduced contamination along the wetlands. However, Copper, Lead and Zinc exceeded wastewater limit values applicable to discharges of wastewater into water resources. Even at low concentrations, heavy metals are a health risk to aquatic organisms and humans. The present study champions Sustainable Development Goals (SDGs) 3: Good health and well-being, SDG 6: Clean water and sanitation and SDG 15: Life on land. Although wetlands are considered natural wastewater treatment entities, wastewater effluent should be effectively treated before discharge into receiving water resources. Furthermore, wetlands should be protected to improve the water quality of receiving surface water environments, which often serve as primary sources of drinking water.

Keywords: Wetlands, Heavy metals, Wastewater, Sustainable Development Goals

1.13.P-We078 Are vulnerable insects sentinel species of chemical mixture threats in small streams? Resolving the debate around the German Kleingewässermonitoring (KGM)

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Pesticide applications on agricultural lands contribute to the input of chemical mixtures into adjacent small lowland streams, where their toxic effects may impair biodiversity and ecosystem services that underpin One Health. A study on 101 small agricultural streams across Germany suggested that pesticides are the dominant stressor for vulnerable insects (Liess et al. 2021 Wat. Res.), whereas a recent study pointed to potential flaws in data analysis (Moore & Rathjens 2025 IEAM). These revolved around non-reproducibility of potential toxicity estimates, temporal mismatch of chemical and ecological monitoring and missing data. Given that the latter study did not resolve the issue of non-reproducibility and did not provide a comprehensive analysis of different decisions in data analysis, we aimed to resolve this issue through a reproducible re-analysis. We compared different methods of estimating potential toxicity in terms of toxic units. We were able to reproduce both the original results and those of Moore & Rathjens (2025). Irrespective of the method, we observed stronger associations between chemical mixtures and ecological metrics compared to individual substances. Moreover, associations with ecological metrics increased when both event-based and grab samples were included, which probably reflects that toxicity can occur during both dry-weather and rainfall periods, with one-third of sites exhibiting higher toxicity in dry-weather samples. When accounting for the temporal mismatch, the results still confirmed the primary hypothesis on negative association of pesticide mixture with sentinel species health. Across the gradient of agricultural land use (<20% to 100% coverage), the estimated potential mixture toxicity from pesticides was the dominant driver of declines in vulnerable insect species for most choices in data pre-processing. Our results demonstrate that the original conclusions of Liess et al. (2021) remain valid even when accounting for the points raised by Moore & Rathjens (2025). We discuss the role of freshwater insects as sentinel species integrating chemical mixture exposures over time and revealing ecosystem-level impacts.

Embedding such field-base evidence into integrated health frameworks might better safeguard freshwater biodiversity, ecosystem services and human health in an age of global pollution.

1.13.P-We079 Comparative Ecotoxicological Assessment of Nano-formulated and Conventional Copper-based Pesticides on Crop Plants, Freshwater Organisms and associated Microbiota: A One Health Framework

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Copper-based nanopesticides are increasingly used in agriculture because they are promoted as more efficient alternatives to conventional copper products due to their improved antibacterial and antifungal properties. Such formulations are applied to crops and can then be transported to adjacent freshwater ecosystems, creating a continuum of exposure for plants, non-target wildlife and associated microbiota. In this context, we evaluated the ecotoxic potential of the copper-based nanopesticide Kocide 3000® compared to the conventional formulation Kocide 2000®, from crop plants to freshwater organisms while considering associated microbiota. Tomato (*Solanum lycopersicum*) plant microcosms were subjected to a realistic foliar application scenario. In parallel, freshwater species representing distinct ecological niches were directly exposed in water to each copper formulation under controlled laboratory conditions. Beyond acute toxicity, we investigated developmental effects using *Chironomus riparius* and *Xenopus laevis* as holobiont models. In the terrestrial compartment, no phytotoxic effects were detected and neither formulation measurably altered the soil bacterial community composition. However, the nanopesticide caused a significant accumulation of copper in tomato fruits, indicating a potential dietary exposure route for humans. On aquatic organisms, analyses showed that all contaminants, induced toxicity at environmentally relevant concentrations (0.005 to 0.050 mg Cu L⁻¹). Regarding microbiota responses, both commercial formulations induced distinct shifts in bacterial taxa. In the gut microbiota of amphibian larvae, only the nanopesticide induced significant changes compared to the negative control at all tested concentrations. Finally, the differential and significant bioaccumulation potential of the nanopesticide in biological matrices suggests a higher risk of copper transfer through aquatic-terrestrial food webs, which could impact biodiversity. Together, these findings question the assumption that nano-formulations are safer alternatives and emphasize the need for further research into the mechanisms underlying the impacts of copper nanopesticides, as well as their broader ecological consequences following agricultural use. Overall, this multi-species, microbiota-informed approach illustrates how commercial pesticides can link environmental contamination, ecosystem health and potential human exposure within a One health framework.

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1.13.P-We080 Combined Low-Dose Toxicities and Interactions of Agricultural Toxicants in *Caenorhabditis elegans*: Epigenetics Insights

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Combined toxicities are increasingly recognized as a critical environmental health concern. Under climate change, alterations in fungal contamination patterns and crop protection practices are likely to elevate the probability of co-exposure to multiple agricultural toxicants. This emerging reality highlights the necessity for continued investigation of toxicant mixtures to support cumulative impact assessment. However, most existing evidence is derived from in vitro assays, leaving a notable lack of long-term in vivo co-exposure data. In addition, potential interactions between toxicants complicate predictions of combined toxicities based solely on individual compound assessments. Given these gaps, a key challenge is determining whether toxicants interact in vivo and identifying the molecular pathways that connect mixture exposure to organism-level adverse

outcomes.

To address this, we applied an integrated experimental-computational approach using *Caenorhabditis elegans* (*C. elegans*). Long-term co-exposure interactions were assessed with the Chou-Talalay method using phenotypic endpoints, with mixture ratios defined by each toxicant's EC10 to analyze chronic interactions. Molecular responses were interpreted within an adverse outcome pathway (AOP) framework, emphasizing epigenetic initiating events associated with sustained low-dose toxicities. Preliminary results indicated that zearalenone (ZEN), a mycotoxin with estrogenic activity, and tebuconazole (TEB), a widely used triazole fungicide, synergistically affected body bending locomotor behavior in *C. elegans* at a 10% effect level. At this exposure level, the concentrations required to elicit comparable deficits were reduced by 2.4-fold for ZEN and 4.0-fold for TEB. The results also demonstrated that co-exposure to ZEN and TEB significantly affected the epigenetic regulatory mechanisms.

These findings indicate that mixture effects cannot be predicted from single-toxicant data alone, and that epigenetic AOP signatures providing a mechanistic basis for interpreting these interactions. The study advances mixture toxicology by delivering long-term in vivo evidence of low-dose co-exposure, a framework for interaction classification, and insights into molecular mechanisms underlying adverse phenotypes. These results can inform regulatory mixture assessments and improve cumulative exposure models for environmental protection.

1.13.P-We081 Synergistic Toxicity Induced by Co-exposure to Zearalenone and Deoxynivalenol in *Caenorhabditis elegans*

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Mycotoxins are fungal secondary metabolites that are widely distributed in the environment and represent a significant global concern for both human and animal health. Among them, zearalenone and deoxynivalenol are commonly detected in grains such as wheat, corn, and barley, in food products and animal feed, and even in human urine. Furthermore, reports of the co-occurrence of zearalenone and deoxynivalenol have been increasing in recent years. Therefore, the interactions between these two mycotoxins, whether synergistic or antagonistic, warrant further investigation.

However, current studies are conducted under varying exposure concentrations and across different species, with inconsistent exposure durations and without rigorous approaches to clearly define synergistic or antagonistic interactions. Based on this background, the present study aims to investigate the interactive effects of developmental and reproductive toxicity induced by co-exposure to zearalenone and deoxynivalenol at various concentration combinations using *Caenorhabditis elegans* as a model organism. The interactions were evaluated with the Bliss independence model and the Chou–Talalay model, as these approaches were more consistent with the assumptions of our experimental design.

The results showed that, in terms of developmental toxicity, co-exposure to lower concentrations of zearalenone and deoxynivalenol (1.25 + 2.5 μ M) exhibited a synergistic effect, whereas higher concentrations (2.5-125 + 5-250 μ M) showed antagonistic effects. Regarding reproductive toxicity, lower concentrations of zearalenone and deoxynivalenol (1.25-25 + 2.5-50 μ M) produced synergistic effects, while higher concentrations (75-125 + 150-250 μ M) exhibited antagonistic effects. We further found that co-exposure to zearalenone and deoxynivalenol (1.25 + 2.5 μ M) also exerted synergistic toxic effects on mitochondrial dynamics, including fusion and fission processes.

In conclusion, these findings suggest a potentially high risk of low-dose co-exposure to mycotoxins. Based on these preliminary results, we will further focus on elucidating the underlying molecular mechanisms to understand how zearalenone and deoxynivalenol synergistically affect the reproductive and developmental systems in *C. elegans*.

1.13.P-We082 Safety Evaluation of Synthetic Compounds for Fire Blight Management: Environmental Fate and Ecotoxicity Assessment

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Fire blight, caused by *Erwinia amylovora*, remains one of the most detrimental diseases affecting global pome fruit production, underscoring the need for effective and environmentally compatible management options. As part of the project “Field Application and Control System Development of Synthetic Compounds for Fire Blight Management,” we assessed the baseline safety profiles of four candidate compounds: pharmaceutical-derived bioactives (Azomycin, Enrofloxacin) and natural product–derived bioactives (Nifuratel, Nifuroxazide).

Partition-coefficient (n-octanol/water, log Pow) analysis indicated that all compounds were generally hydrophilic, suggesting minimal potential for environmental bioaccumulation. Enrofloxacin showed comparatively higher lipophilicity, which may contribute to enhanced surface persistence and potential field efficacy. Acute aquatic toxicity tests using *Danio rerio* revealed moderate toxicity for Azomycin and Nifuratel, and low toxicity for Enrofloxacin and Nifuroxazide. Biodegradability assays demonstrated partial degradability only for Nifuroxazide. Skin-irritation tests confirmed that all four compounds were non-irritants, indicating a favorable safety profile for agricultural handlers.

Collectively, these findings highlight that the candidate compounds exhibit low bioaccumulation potential and low-to-moderate aquatic toxicity, supporting their suitability for further development as fire blight control agents. Additional studies on chronic toxicity, soil microbial interactions, and environmental persistence are recommended to ensure comprehensive safety evaluation and sustainable application.

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1.13.P-We083 Tier 1 Ecological Risk Assessment of Sulfa Drug Mixture Discharges from Livestock Facilities in Japan Using an RPF-Based Approach

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In recent years, the discharge of antimicrobial agents from livestock facilities into rivers has attracted considerable attention, as there are growing international efforts to substantially reduce the use of antimicrobials in agricultural and food systems in order to address environmental concerns. Sulfa drugs are among the most widely used veterinary antimicrobials in Japan, representing a diverse group of compounds with similar pharmacological and toxicological modes of action (MoA). Despite their extensive use, there is still insufficient monitoring data on environmental concentrations of sulfa drugs, and the actual discharge patterns from livestock operations remain poorly characterised.

This study aimed to evaluate the magnitude and possibility of ecological risks to aquatic organisms associated with the release of sulfa drugs into the environment from livestock facilities in Japan. To achieve this objective, it was necessary to assess the combined risk of multiple sulfa drugs with similar MoA that may be released simultaneously. Therefore, a Tier 1 risk assessment was conducted based on the WHO/IPCS framework for assessing combined exposure risks from multiple chemicals.

To estimate environmental concentrations, a simple yet transparent method was adopted: the discharge amount of each drug in each river segment was predicted using national sales data combined with available information on drainage volumes from individual farms. A higher-tier method with lower uncertainty - the relative potency factor (RPF) approach - was applied to assess the hazard. The RPFs of the sulfa drugs were estimated using a regression equation obtained from trend analysis of reliable ecotoxicity values and parameters related to the MoA. The risk assessment incorporated some biological metabolic processes.

The results of the Tier 1 assessment indicated that the combined exposure to the sulfa drug mixture revealed higher ecological risks than those identified in single-compound assessments. These findings emphasise the importance of monitoring water quality to identify all sulfa drug hotspots, and highlight the potential need for improved wastewater treatment for livestock facilities to mitigate the environmental risks associated with the mixture of sulfa drugs.

1.13.P-We084 A NAMs Approach for Hazard Assessment of Leachates of Environmentally Relevant Plastic Mixes Collected in the Southeastern Bay of Biscay

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Despite increasing micro and nanoplastic (MNP) research, limited information exists on toxicity of realistic environmental MNPs and their risk to human and environmental health. This is of special relevance in accumulation areas such as the Bay of Biscay. This study aims to assess the toxicity of environmental MNP mixes representative of plastics collected in beaches of the Southeastern Bay of Biscay and of MPs collected in

a SeaBin located at the port of Pasaia on selected fish and human cell lines. Beached plastics were collected once per season (2023-2024) in 4 beaches of the Bay of Biscay (Gorronatxe-Getxo, Alkolea-Mutriku, Murgita-Donostia and Tarnos-Baiona). Polymer composition of each item was identified by FTIR and items made of the same polymer were micronized up to <250 µm. Micronized polymers were mixed keeping the polymer proportion by weight to produce a MNP mix representative of each beach and all beaches (Bay of Biscay mix). For the SeaBin, MPs were collected during 1 week per month (March 2023-March 2024) using a 500 µm net. The MPs were sorted using milimetric paper and divided into 5 morphological categories. Polymer composition of 100 items per category was identified by FTIR. For toxicity assessment, filtered distilled water was added to each mix at 10 g/L, agitated (24h, 190 rpm, 18°C, darkness) and filtered (0.8 µm). Human intestinal Caco2 cells and fish RTL-W1 and RTG-W1 cells were exposed to 20-100% dilutions of each leachate for 24 h and cytotoxicity was assessed (Alamar or Deep blue). Then, cells were exposed to the first sublethal dilution to assess ROS production, integrity of the plasma membrane (PMI) and/or EROD activity. Metal and organic compounds were measured and the presence of NPs was checked by SEM. The Bay of Biscay and SeaBin mixes consisted mainly of polyethylene (PE), polyvinyl chloride, and polypropylene (PP) and PE, polystyrene and PP, respectively. Lead, phthalates and alkylphenols were detected in all leachates. By SEM, NPs were abundant in leachates from Gorronatxe and Bay of Biscay mix. For all cell lines, leachates of Bay of Biscay mix and SeaBin significantly decreased cell viability at 100% dilution. All leachates decreased PMI in Caco2 cells and increased ROS and EROD activity in fish liver cells. These results highlight the usefulness of NAMs approaches for toxicity profiling of complex mixtures such as environmental MNPs in order to assess their risks on human and environmental health.

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1.13.P-We085 Longitudinal Differences in Microplastics in Amniotic Fluid between Mid- and Late Pregnancy and Exploratory Assessment of Perinatal Outcomes

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Microplastics (MPs) have been increasingly detected in human biological samples, raising concerns about fetal exposure and potential impacts on pregnancy health. While previous studies have identified MPs in amniotic fluid and placenta, data describing gestational changes in intrauterine MP burden remain limited. Given that MPs may accumulate over time and potentially influence fetal environment, understanding how MP profiles differ across gestation is essential.

This study aims to evaluate how characteristics of MPs in amniotic fluid change from the mid-trimester (14-28 weeks) to the late-trimester (>28 weeks). Additionally, we explore whether MP burden is associated with pregnancy outcomes.

Under appropriate IRB approval and informed consent, amniotic fluid was obtained during clinically indicated mid-trimester amniocentesis and at cesarean delivery in late pregnancy. To prevent plastic contamination, all procedures were performed exclusively with cotton, glass, and metal instruments. 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 to evaluate gestational-age-related differences in intrauterine MP exposure. Pregnancy outcomes – including gestational age at delivery, birthweight, and neonatal clinical parameters – were explored in relation to MP burden. This study provides foundational evidence on temporal patterns of MPs in amniotic fluid and offers preliminary insight into their potential relevance to perinatal health.

1.13.P-We086 Assessing Chemical Pollution Through an Invasive Species: *Procambarus clarkii* as a Bioindicator in the Avigliana Lakes

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Invasive alien species are increasingly recognised as valuable bioindicators in environmental chemistry due to their high abundance, wide distribution, and resilience in contaminated habitats. The red swamp crayfish (*Procambarus clarkii*), a major invasive species in European freshwaters, has recently emerged as a promising model organism for assessing chemical pollution. This study investigated the occurrence of persistent and emerging contaminants in *P. clarkii* collected from the Great Lake of Avigliana (northern Italy), with the aim of evaluating its suitability as a freshwater bioindicator.

During the summer seasons of 2024-2025 crayfishes were captured using baited cylindrical creel traps. Individuals were euthanised by hypothermia and dissected to obtain muscle tissues for chemical analyses. Polycyclic aromatic hydrocarbons were quantified using HPLC-fluorescence detection; pesticides were analysed using GC-MS/MS; and rare earth elements (REEs) were measured via ICP-MS. Microplastics were extracted from the gastrointestinal tract and identified using micro-FTIR.

Among PAHs, only chrysene was detected in muscle tissue, with a mean concentration of $0.9 \pm 0.2 \mu\text{g/kg w.w.}$. All pesticide residues were below the limit of quantification. Mean total REE concentration was $0.0046 \pm 0.0061 \text{ mg/kg w.w.}$, with cerium and yttrium being the most abundant elements ($0.0215 \pm 0.0059 \text{ mg/kg}$ and $0.0117 \pm 0.0031 \text{ mg/kg}$, respectively). Microplastics were present in several individuals, ranging from 1 to 6 particles per crayfish, dominated by blue, black, and white fibres and fragments composed primarily of PET, PE, and PP. Particle sizes ranged from approximately 120 to 450 μm .

Chrysene is classified as a possible human carcinogen, and studies on other crustaceans indicate that concentrations in the microgram range may affect neuroendocrine and immune functions. In Italy, the regulatory limit for chrysene in soils is 5 mg/kg d.w. for agricultural areas, while only groundwater limits (5 $\mu\text{g/L}$) currently exist for freshwater systems. The ecotoxicology of REEs remains poorly understood; available studies report sublethal effects such as oxidative stress and reproductive impairment, potentially influencing growth and population dynamics. Overall, these results support the use of invasive crayfish as practical, informative, and ecologically relevant bioindicators for monitoring freshwater contamination, particularly in systems where native species are declining.

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1.13.P-We087 The Potential of Invasive Alien Species to Serve as Bioindicators of Chemical Pollution

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Invasive alien species (IAS) are increasingly recognized as valuable tools for assessing environmental pollution, complementing their better-known role as drivers of ecological change. Their broad distribution, resilience to stressors, and frequent dominance in degraded habitats make them suitable candidates for biomonitoring, particularly where native species are declining or protected. Recent studies show that IAS across multiple taxonomic groups, including plants, bivalves, crustaceans, fish, and mammals, can accumulate diverse contaminants such as trace metals, persistent organic pollutants, pharmaceuticals, PFAS, pesticides, and microplastics. Evidence from the last two years demonstrates that species like *Dreissena polymorpha*, *Corbicula fluminea*, *Procambarus clarkii*, *Lepomis gibbosus*, and *Myocastor coypus* reliably mirror local pollution patterns and in some cases support broad-spectrum contaminant monitoring. Despite this potential, several limitations constrain their wider implementation. Physiological and ecological differences between IAS and native species may lead to divergent bioaccumulation patterns, complicating risk assessments and reducing comparability across ecosystems. Furthermore, environmental variability, regulatory frameworks, and the ethical implications of relying on invasive populations require careful evaluation. Standardized protocols and integrative approaches, linking chemical analyses, biomarker responses, and molecular tools, are essential to ensure robust and ecologically meaningful applications. When incorporated into management or control actions, IAS may

simultaneously support pollution monitoring and contribute to invasive species mitigation. Overall, IAS represent an emerging yet underutilized resource for environmental chemistry, with strong potential to enhance contaminant surveillance while minimizing pressure on vulnerable native species.

1.13.P-We088 River water contaminated by a “cocktail” of chemicals: antimicrobial resistance and estrogenic effects

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River waters can contain a mixture of legacy and emerging pollutants, including pharmaceuticals, and in particular antibiotics, which are of significant concern due to their potential impact on ecosystems and human health. These contaminants can derive from wastewater treatment plant (WWTP) discharges and may cause the disappearance or inhibition of specific microbial species involved in key ecosystem functions, while also promoting the spread of antibiotic resistance in natural microbial populations.

The Danube river was sampled over a 3-year period (2023-2025) in 2 seasons (spring and autumn) at selected sites inside and outside Budapest city. Various compounds were analysed, including antibiotics, metals, antipyretics, anti-inflammatories, hormones, anticonvulsants, insect repellents and disinfectants. The presence of several antibiotic resistance genes (ARGs) was also investigated. Finally, in line with the new Directive on water quality (COM(2022) 540 final), potential estrogenic effects were evaluated in river water samples using the Yeast Estrogenic Screen (YES) test.

Overall, the results showed diffuse pharmaceutical residual concentrations (e.g. antibiotics, antipyretics and anti-inflammatories) in all sampling points, with seasonal differences. Sulfamethoxazole and ciprofloxacin were the antibiotics detected at the highest concentrations in all three sampling points. ARGs were consistently present, with the highest levels associated with sulphonamides, although their occurrence was not directly correlated with antibiotic concentrations.

Because some estrogenic effects were observed in river water samples without a clear correlation to hormone concentrations, we further evaluated the estrogenicity of selected compounds (diclofenac, ibuprofen, paracetamol, acetylsalicylic acid), both individually and in mixtures at concentrations matching those detected in the river. Estrogenic activity of these substances, when tested individually, was observed only at high concentrations (250-1000 pM). However, when assayed in mixtures at low concentrations similar to those detected in the river water samples, they exhibited cumulative estrogenic activity. The estrogenic responses observed for the chemical mixtures highlight the importance of assessing this aspect in water samples, and suggest that estrogenicity may also be influenced by pollutants other than hormones, even when present at residual concentrations

1.14.P Navigating the Challenges of Difficult-to-Test Substances in Ecotoxicology and Environmental Fate Assessment

1.14.P-Mo075 How to Perform REACH Studies in a Smart Way? A Case Study with Industrial Chemicals on Aquatic Systems Highlighting Challenges and Solutions

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REACH-compliant ecotoxicological testing focusing on aquatic environments explores challenges and innovative strategies when conducting studies. The shift from testing plant protection products to a broader array of REACH-regulated substances has introduced significant analytical complexities. Unlike the simpler, smaller molecules traditionally analyzed for Plant Protection Products, many REACH substances are complex, poorly soluble, unstable or volatile, requiring adaptations in test setups and analytical methodologies.

The key challenges highlighted include difficulties in preparing stock solutions, maintaining accurate dosing, and handling substances that exhibit problematic behaviors such as biofilm formation, volatility, or instability. Traditional analytical methods (e.g. TOC analysis) are inadequate and proven to be insufficient under the stringent requirements of REACH regulations. Enhanced analytical techniques, such as HPLC, GC-MS, and LC-MS/MS are necessary for precise quantification, especially at very low concentrations.

To overcome these issues, a “smart testing” approach is advocated. This involves conducting feasibility studies

before the main biological assessments, leveraging in-depth analysis of the chemical properties to tailor testing methods. Emphasis is placed on method development, application approaches, enhanced communication and collaboration between regulatory bodies and testing facilities. The goal is to optimize test systems, reduce unnecessary studies, and ensure compliance while maintaining high standards of scientific rigor.

Adaptive testing strategies and robust analytical capabilities are important to meet the evolving requirements of REACH regulations, ultimately ensuring the environmental safety of industrial chemicals.

1.14.P-Mo076 Tackling pH-dependent Toxicity of Ionisable Organic Chemicals in Aquatic Risk Assessment

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The characterisation of the toxicity of ionisable organic chemicals (IOCs) and related environmental risk assessments come with uncertainties, as the toxicity of IOCs towards non-target organisms can strongly vary depending on the physico-chemical properties of IOCs, their mode of action, the pH of the aqueous test medium, and the organism. Hazard endpoints of regulated chemicals from toxicity testing at specific pH values (of the test medium) can over- or underestimate the toxic potential of IOCs across environmentally relevant pH ranges (in soil and water) by up to three orders of magnitude. However, technical possibilities for testing or mathematical prediction of the actual toxicity of IOCs to non-target organisms are limited (as e.g. ecotoxicity testing may not be possible across wide pH ranges, while universal, generally accepted prediction models are not available). Consequently, in regulatory practice, pH-dependent toxicity of IOCs to non-target organisms is either subtly ignored or arbitrarily tackled by unrealistically high safety factors derived from simplified prediction models.

This case study evaluates the extent of pH-dependent differences of the toxic potential of IOCs in aquatic species and the relevance of generalised predictions of the aquatic toxicity of IOCs at different pH values. A dataset was compiled from peer-reviewed literature summarising endpoints from approximately 50 acute tests on the toxicity of IOCs (incl. pharmaceuticals, pesticides, industrial chemicals, and biocides) to fish, invertebrates, algae, and macrophytes, which were conducted in test media with varying pH. For each IOC, toxicity data determined experimentally at a selected pH value were used to predict the toxicity of the IOC at other pH values, and the model predictions were subsequently compared to corresponding experimental effect data (at the same pH). Our results show the suitability of the different models for generalised prediction of pH-dependent toxicity of IOCs in aquatic organisms. Additionally, it is discussed how ‘potential outliers’ (i.e., IOCs that were not adequately captured by the models) can be tackled using current OECD test protocols to ensure a protective aquatic risk assessment. The outcome of this case study highlights strengths and weaknesses of generalised predictions of the pH-dependent toxicity of IOCs and thus supports the improvement of aquatic risk assessments.

1.14.P-Mo077 Are fatty alcohols ‘difficult test substances’? Exploring the downsides of rapid biodegradability in combination with poor water solubility.

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Fatty alcohols are a class of long-chain aliphatic alcohols that are commonly used as intermediates in the production of other common-use substances (e.g., surfactants) or as lubricants, process chemicals, laboratory chemicals, and solvents in commercial and industrial applications. Derived from petrochemicals, natural fats or oils, these substances are widely sold and manufactured - often registered under REACH at high tonnages (> 1000 tonnes per annum) in the European Union. Available environmental fate studies have demonstrated that these substances are highly biodegradable, with all registered C6 – C24 alcohols being classified as ‘readily biodegradable’. Further, available simulation studies demonstrate rapid mineralization in freshwater, sediment, soil, and sewage treatment plants. Rapid biodegradability is a desirable property for industrial and commercial chemicals as it lowers the environmental risk profile of a substance. However, this environmental fate behavior also introduces significant challenges for ecotoxicity testing. Moreover, when this property is combined with other inherent properties of these substances (e.g., poor solubility) – fatty alcohols can become very ‘difficult to test’ under chronic and acute exposure scenarios.

This poster aims to present some major challenges associated with conducting aquatic toxicity testing with fatty alcohols. Highlighting issues encountered during short- and long-term aquatic toxicity testing conducted with diverse taxa, we demonstrate how properties such as rapid biodegradability and poor solubility can introduce significant challenges when conducting these tests according to currently available guidance. We further discuss

successes and failures with adapted or alternative test methods, building evidence that can be used to inform future testing plans and strategies for similar substances. Finally, we put available test data in the context of environmental risk – raising the question of whether fatty alcohols are indeed ‘difficult test substances’ or whether toxicity testing requirements for such substances (e.g., highly biodegradable, potentially poorly soluble) should instead be adapted to better reflect likely environmental exposure scenarios.

1.14.P-Mo078 Beyond Dissolved Fractions: Rethinking Toxicity Assessment of Cationic Surfactants in Algal Test Systems

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Cationic surfactants, particularly primary fatty amines (PFAs), present unique challenges in ecotoxicological testing due to their surface-active properties and strong adsorption tendencies. Traditional approaches to toxicity assessment rely on measured dissolved concentrations, with regulatory authorities increasingly demanding evaluation based solely on truly dissolved fractions. However, our research demonstrates this approach is fundamentally flawed for cationic surfactants in aquatic systems especially when algae or other organic matter are present (specifically alga toxicity and chronic daphnia studies). Using a radiolabeled PFA, we tracked substance distribution throughout an algal growth inhibition test, revealing that a significant amount of the total substance becomes bound to algal cells within 72 hours. As algal density increases during study duration, substance, initially bound to the glass surface, transfers from glass surfaces to algae, creating a dynamic redistribution system. Additional studies with non-radiolabeled PFAs of different chain-lengths confirmed that centrifugation, used to isolate truly dissolved fractions, introduces further methodological artifacts through adsorption to equipment surfaces, which is demonstrated in both types of study. Since cationic surfactants mainly exert toxicity through membrane disruption, the algae-adsorbed fraction actively contributes to observed effects rather than reducing its bioavailability. Both the concentration at the organism surface and in the surrounding medium significantly impact toxicity, with their combined effect determining the overall toxicological outcome. Our findings challenge current regulatory paradigms and demonstrate that for cationic surfactants, nominal concentrations based on carefully designed water-accommodated fractions provide more reliable metrics for toxicity assessment than truly dissolved concentrations, which substantially overestimate toxicity by underrepresenting the actual bioavailable amounts.

1.14.P-Mo079 Dosing of very hydrophobic chemicals in high-throughput microtiter plate-based in vitro bioassays

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New Approach Methodologies (NAMs), including in vitro bioassays, have transformed and advanced next-generation chemical hazard and risk assessment. However, current in vitro bioassays predominantly operate within a narrow physicochemical window, mostly covering chemicals of moderate hydrophobicity (octanol-water partition constant $1 < \log K_{ow} < 5$). Hydrophobic chemicals ($\log K_{ow} > 5$) often face technical testing barriers such as solubility issues or precipitation in the microtiter-plate-based in vitro assays, resulting in frequent “nontoxic” outcomes simply because biologically effective doses in the cells cannot be reached. Serum-mediated passive dosing (SMPD) helps stabilize hydrophobic chemicals in small volume cell-based in vitro assays by supplementing the bioassay medium with colloids. Sorption to colloids increases apparent solubility, and the chemicals can desorb from the colloids and enter cells without depleting freely dissolved concentrations. The presence of colloids accelerate the uptake kinetics in the static exposure of microtiter (96- and 384-well) plates. However, SMPD still fails for (super)hydrophobic substances due to precipitation with floating microcrystals, which can be avoided by passive dosing into medium before dosing the saturated medium into bioassays. By swelling the silicone with a solvent solution of the chemical and evaporating the solvent, the chemical can be enriched over its PDMS solubility. This creates an oversaturated silicone containing microcrystals inside. During passive dosing, dissolution of these microcrystals replenishes medium concentrations, allowing the medium to be dosed at maximum solubility. This highest concentration in medium can then be serially diluted and applied to cells in microtiter plates. With sufficient serum protein, cellular uptake does not visibly deplete medium concentrations. We demonstrate this approach with a range of phenolic benzotriazole UV stabilizers and brominated flame retardants. It was possible to dose hydrophobic chemicals up to a octanol-water partition constant $\log K_{ow}$ of 8 but equilibrium was not reached for more hydrophobic

chemicals, indicating further optimization on the passive dosing is needed for chemicals with logKow > 8. The method is versatile and can be adopted to any cell exposure set up and is suitable for bioassay testing in high-throughput format, although sample preparation remains work-intensive.

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1.14.P-Mo080 Addressing chronic aquatic toxicity test requirements for difficult to test surfactants

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ERASM (Environment and Health – Risk Assessment & Management) commissioned a project to gather and synthesize data on properties which make surfactants difficult to test in chronic aquatic toxicity tests and solutions to optimise test performance. Data were collected through a search of published literature, including the Organisation for Economic Cooperation and Development (OECD) Test Guidelines and Guidance Document 23, and questionnaires were completed by contract research organisations (CROs) and industry representatives on their experiences with difficult to test surfactants.

Many surfactants require chronic aquatic toxicity testing under European Union (EU) Registration, Evaluation, Authorisation and restriction of Chemicals (REACH), but are difficult to test due to their surface activity. Many are also UVCBs (substances of Unknown or Variable composition, Complex reaction products or of Biological Origin), for which specific adaptations are necessary. Other key challenges include: poor water solubility, adsorption, rapid degradation, ionisation across the environmental pH range, volatility, and/or high background contamination. These challenges can mean ecotoxicity data may not meet reliability criteria or are difficult to interpret for regulatory purposes.

Adaptations for testing surfactants were considered based on: optimising solubility and dosing methods, modifications to the test design, adaptations based on the analytical method and reporting of results, and whether alternative ecotoxicology tests might be appropriate. Our findings show direct addition to be the most common dosing method, and most CROs avoided emulsions, solvents and generator systems. Potential modifications were identified to avoid formation of surface films and aggregation of particles, including changes to test vessels, test media and feeding regimes. Alternative tests, such as a 7 day Ceriodaphnia study instead of a 21 day Daphnia study, and waivers were also considered.

This review collects examples of potential solutions to challenges for chronic aquatic toxicity testing of difficult to test surfactants, in order to understand the feasibility, applicability and regulatory acceptability for future testing. The review identifies gaps in the available guidance and areas where challenges remain with testing surfactants. It also notes where potential solutions from guidance are not adopted.

1.14.P-Mo081 Beyond Standard Protocols: Chronic Fish Toxicity Assessment of UVCBs

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Under EU REACH, regulatory authorities mandate long-term toxicity studies for poorly soluble substances to account for effects occurring at equilibrium. This poses challenges specifically for chronic fish toxicity testing as adherence to the 3R principle (Replace, Reduce, Refine) for animal testing is crucial. Generating reliable chronic data for Difficult-to-Test Substances (DTTS) and Substances of Unknown or Variable composition, Complex reaction products, or Biological materials (UVCBs) is challenging, as stable exposure is difficult to maintain. To address this, a battery of pre-tests is performed to optimise methodology. However, for registrants, the extent of work required to confirm technical feasibility can be difficult to assess. This submission aims to provide insightful discussions on the possibilities and limitations of chronic fish toxicity testing with poorly soluble UVCBs.

This submission will examine the testing strategies for two chronic fish toxicity studies (OECD 210) involving two very poorly soluble UVCBs with challenging properties (e.g., hydrolytic instability, adsorptivity, surface activity). The core strategy for both involved analytical method development and detailed ECHA-recommended pre-tests to determine solubility, stability, and optimal application, maximising success (Refine) and minimising animal use (Reduce).

For one poorly soluble UVCB, the full study was technically infeasible and could not be initiated due to

analytical limitations in accurately determining a stable, truly dissolved exposure concentration. For the second UVCB, successful stable exposure required strategic protocol adaptations informed by extensive pre-testing, including precise application control and rigorous analytical monitoring.

In conclusion, chronic fish toxicity testing of poorly soluble UVCBs requires significant upfront investment in adaptive methodology and analytical strategy. Although ECHA-guided adaptations and comprehensive pre-testing may improve success, our experience indicates that analytical limitations can render a chronic study scientifically unjustifiable and technically infeasible for some substances. These case studies underscore the need for increased regulatory guidance and acceptance of innovative testing approaches under REACH to better predict effort, assess feasibility, and improve the quality and regulatory acceptance of future chronic fish toxicity studies, thereby supporting regulatory toxicology and the 3R principle.

1.14.P-Mo082 Adapting the Fish Embryo Toxicity Test to Overcome Limitations in Aquatic Testing of Anticoagulant Rodenticides

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Anticoagulant rodenticides (ARs) used for large-scale rodent eradication measures can contaminate small surface water bodies in particular following aerial dispersal of baits for wildlife conservation but also by accidental spills, leading to waterborne exposure of wild fish with potentially toxic effects. To date, studies on sublethal and lethal effects of ARs on fish are limited, especially regarding effects not directly linked to the known mechanisms of action of ARs. One reason is that ecotoxicity tests with ARs in fish are challenging due to their physicochemical properties such as low water solubility, adsorption, photolytic sensitivity, and potential ionizability. Another challenge is that their specific mode of action causes a delayed onset of symptoms. In our study, we aimed at overcoming some of the limitations of AR testing in aquatic species to further explore adverse effects of ARs on fish. Hence, zebrafish (*Danio rerio*) embryo toxicity tests were conducted with different AR active ingredients according to the OECD236 test guideline and extended by the assessment of sublethal morphological effects. Notably, substance specific adaptations were required to facilitate the application of these difficult-to-test substances. The exposure concentrations in the 96-multiwell-plates ranged from 0.1 to 7 mg/L. Aqueous AR concentrations in individual wells were monitored by LC-MS/MS and generally, showed good recovery of at least 80% after 96 hours in all treatment groups. While coumatetralyl and bromadiolone were equally toxic to the zebrafish embryos, observed morphological malformations varied, from chorda deformations and reduced head development to vascular defects and no heartbeat. For all tested ARs, a sudden increase in mortality was detected after hatching of the embryos, possibly indicating a protective function of the chorion. Effect thresholds (EC20-values) exceeded available predicted environmental AR concentrations in surface water. Yet, chronic effects must be considered due to the bioaccumulative properties of second-generation ARs. Therefore, we also investigated whether the known mechanistic effects of the tested ARs are linked to the developmental effects occurring in zebrafish embryos, and whether these early effects could substantiate evidence for presumed adverse outcomes for wild fish.

1.14.P-Mo083 The AFSA DIM Testing Project: When Animal Testing is Difficult, Impossible and Meaningless (DIM) and Implications for the REACH Regulation

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The EU's REACH Chemicals Regulation Article 25 mandates that animal testing should be used only as a last resort. Adaptations of this standard (i.e. using animal test models) testing regime are described primarily through Annex XI. However, traditional in vivo testing remains common, often due to procedural rigidity and/or lack of understanding or proper application of regulatory framework, rather than scientific necessity. For regulatory ecotoxicological endpoints under industrial chemical legislation, an average of 21,070 animals per year have been used according to the ALURES Statistical EU Database.

The AFSA Collaboration launched a multi-phase project focusing on animal tests that are Difficult, Impossible, or Meaningless (DIM). DIM testing refers to cases where in vivo studies have been performed or requested when they are scientifically unfit, unjustified, technically infeasible, or irrelevant to real-world safety outcomes. Such tests often yield redundant, unreliable or unusable results, particularly for substances with physical-chemical properties that compromise standard protocols.

In this project we systematically reviewed registration dossiers and compliance decisions to quantify the prevalence of DIM tests identifying missed opportunities for scientifically sound, non-animal alternatives. The multidisciplinary expert group defined objective DIM criteria for substances (to be) tested and applied them to case studies across sectors, revealing recurring gaps in guidance clarity, methodological relevance, and procedural flexibility.

Initial evidence suggests that ecotoxicological endpoints seem to be most affected, with high animal use and limited regulatory value. Based on these insights, the project has formulated preliminary recommendations to improve the implementation of the last resort requirement, increase regulatory acceptance of novel methods, and clarify when standard animal tests are inappropriate. These include fostering collaboration between registrants and regulators, promoting substance-tailored and exposure-relevant testing strategies, strengthening the integration of toxicokinetics, ensuring scientific robustness across all data sources, and enhancing transparency and traceability of regulatory data.

This work highlights opportunities for a regulatory shift towards relevance-based, exposure-led safety assessment, rooted in robust non-animal methods while maintaining high standards for human and environmental protection.

Disclaimer/Disclosure:

The Animal-Free Safety Assessment (AFSA) Collaboration is a unique multi-sector partnership between non-profit, corporate, and philanthropic leaders working to advance acceptance and use of animal-free safety science as a gold standard across regulatory frameworks worldwide.

1.14.P-Mo084 Challenges Associated with Testing Calcium Fatty Acid Grease Thickeners for EU REACH *Lucy Kennelly, Rhianon Smith, Kate Roylance and Owen Green, wca environment Ltd., United Kingdom*

Calcium fatty acid salts are used as thickening agents for grease products, and are registered as a category under EU REACH (EC 1907/2006 on the Registration, Evaluation, Authorisation and restriction of Chemicals). The substances comprise monoconstituent substances and substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials (UVCBs). A comprehensive testing programme is currently being conducted on this category in order to fill data gaps in the REACH dossiers. Calcium fatty acid salts are classed as difficult-to-test substances, and several associated challenges have arisen during the testing programme.

Calcium fatty acid salts have very low water solubility, which caused issues with obtaining suitable test solutions. For in vitro testing, difficulties were encountered with acquiring dosable solutions for some tests, as the test items were insoluble in all vehicles compatible with the test systems. The use of alternative vehicles is being investigated, including those not typically used in in vitro tests. These issues were encountered during human health genotoxicity testing; however it is anticipated that similar issues will occur for environmental in vitro tests that may be conducted in the future.

Issues have also arisen with developing suitable analytical methods for algae testing. As the test items are salts, an analytical method could not be developed for whole test items, so analysis was performed separately on the calcium and fatty acid components. The calcium component cannot be analysed due to high levels of calcium in the testing medium preventing the acquisition of meaningful results. Calcium is an essential element for algal growth so cannot be removed from the medium. For the fatty acids, difficulties arose in lowering the limits of detection (LOD) and quantification (LOQ) due to high background levels. Quantifiable levels for two substances could not be obtained, and therefore total organic carbon (TOC) analysis is included as a surrogate for fatty acid content but the low solubility means the concentrations may be <LOQ of the TOC analysis. This testing is currently ongoing so further updates will be included in the poster but nevertheless, this case study demonstrates some of the challenges associated with ecotoxicity testing of difficult-to-test substances, and some alternative strategies which can be applied to overcome these challenges.

1.14.P-Mo085 Difficulties with aquatic ecotoxicity testing of substances with constituents present in standard media: A case study with lithium fatty acid salts

Rhiannon Smith and Sylwia Kosmala-Grzechnik, wca environment Ltd., United Kingdom

Under EU REACH (EC 1907/2006 on the Registration, Evaluation, Authorisation and restriction of CHemicals), long-term toxicity testing on aquatic invertebrates is required if the substance has a water solubility <1 mg/L. The standard test for this endpoint is a 21-day *Daphnia magna* reproduction test following the OECD 211 test guideline.

The media recommended for the study are Elendt M4 or M7 media, both of which contain nutritional trace elements for the health of the organisms, which can impact the feasibility of analysis when testing substances which are, or contain, those same trace elements.

A case study is presented for various lithium fatty acid salts, where challenges were encountered with analysis during testing because lithium is present in the test media at 0.05 mg/L.

As the test items are salts, an analytical method could not be developed for the whole test items, so analysis was performed instead on the individual components of the test items, lithium and fatty acids. An analytical method was developed for the fatty acids, but it was not sensitive enough to measure them at the concentrations in *Daphnia* reproduction tests. The concentrations are also below the limit of detection for measuring total organic carbon as a surrogate for fatty acid content. Therefore, analysis focused on the metal.

Analytical methods are available for lithium (inductively couple plasma mass spectrometry; ICP-MS), but the metal is only a small proportion of these test items, so the relative lithium concentration is low at the concentrations used in the OECD 211 studies (concentration range of 0.020 to 0.045 mg/L Li at 1 mg/L nominal concentration of the test items; range reflects multiple lithium fatty acid salts). This means that the lithium concentration from the test items is difficult to distinguish from the background concentrations in the test media. As such, alternative methods are being investigated for testing and analysis, including use of modified or alternative test medium. One alternative being considered is ISO medium, which does not contain lithium as a trace element; however, as is not a standard medium for long-term testing with *Daphnia*, its suitability for the study needs to be confirmed. Therefore, trials are ongoing to culture *Daphnia* in ISO media to ensure suitable conditions before any testing is undertaken.

This case study demonstrates some of the challenges and strategies associated with ecotoxicity testing of difficult to test substances.

Disclaimer/Disclosure:

We would like to thank the European REACH Grease Thickeners Consortium (ERGTC) for allowing their work to be shared.

1.14.P-Mo086 Method Development Procedures for the Analysis of Complex Industrial Chemicals and UVCBs in Support of 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 nature of these substances presents numerous challenges when also providing support for various 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. Developing suitable analytical methods requires addressing several key considerations. 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. If possible, the development of a method that can monitor several marker components is desirable, to provide a more thorough representation of the test material.

A key challenge is selection of the most appropriate detection system that is sensitive enough to quantify the

required analyte concentration in the test samples for the supported studies. It may be that further method procedures are also required, such as sample concentration, but optimization of these steps is often difficult due to the unknown composition of the material as well as the chemical properties of the components that can be quantified.

This poster describes the typical stages of the development of an analytical method for the analysis of a complex chemical test item. It shows the approaches taken and the decisions made, demonstrating the step-by-step procedure of establishing a quantitative method, suitable to reliably quantify test samples generated in ecotoxicology studies.

1.14.P-Mo087 Enhancing ERAs for High LogP Substances: Partitioning to Air (KO/A), Cell membrane (KM/W), and Bovine serum albumin (KBSA/W)

Philip Rooney and Danny Wensley, Smithers, United Kingdom

Partition coefficients in octanol and water provide a rudimentary and overly generic value to describe a substances hydrophobicity. The endpoint (logPOW) of an OECD 107, 117 or 123 study is incorporated into modelling assessments to predict the behaviour of a substance in a wide range of environmental and biological scenarios; examples include its affinity to soil, sediment or sludge, the rate of transfer to water resources or bioaccumulation and elimination within a biological system. To varying degrees, LogP values derived from these regulatory studies are a fundamental, yet superficial input for persistent, bioaccumulation, mobility and toxicology assessments.

The environmental and human impact of emerging contaminants such per- and polyfluoroalkyl substances (PFAS), including PFOA, PFHxS and TFA with more extreme properties ($t_{1/2} < 12$ h, $\log\text{POW} > 6$ or $p_{\text{vap}} > 1$ kPa) require risk assessment and evaluation. The behaviour of these substance classes, deemed ‘difficult-to-test’, is not fully captured by conventional OECD guidelines. This poster details the development of bespoke GLP compliant partition co-efficient studies that investigate partition behaviour across three environmentally and biologically relevant environments: partition between air (KO/A), cell membrane (KM/W), and bovine serum albumin (KBSA/W).

These three coefficients are interconnected and work together to determine the overall distribution of a substance within a macro-scale environment and biological context.

1.14.P-Mo088 Navigating In Vitro Testing Challenges for Complex Hydrocarbon Substances: Improving Dosing and Exposure Characterization

Daniela Holland¹, Ming Kung², Megan Ruhmel², Aaron Redman², Biyao Han³ and Bryan Hedgpeth⁴,

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High-throughput in vitro (eco-)toxicology is central to next-generation risk assessment, yet substances with poor water solubility remain difficult to test reliably. Hydrophobic chemicals, including polycyclic aromatic hydrocarbons (PAHs) and complex hydrocarbon mixtures, may partition to media constituents and plastics, causing unstable exposure concentrations. This undermines conventional dose-response analyses based on nominal concentrations and complicates in vitro-to-in vivo extrapolation (IVIVE).

This work addresses the challenge of achieving reproducible and well-characterized exposures for hydrophobic substances in in vitro test systems that cannot be achieved/maintained using common media-miscible solvents such as DMSO. The influence of dosing strategies on bioactivity outcomes for PAHs tested individually and within a complex hydrocarbon substance was evaluated, by combining in vitro bioassays with partitioning modeling and analytical chemistry. The work showed that standard dosing techniques employed here often fail to maintain steady exposure, leading to variability and underestimation of bioactivity. Results demonstrate that conventional in vitro dosing approaches are inadequate for complex hydrocarbon substances with difficult to test properties. We identified opportunities for methodological refinement to stabilize exposure and accurately quantify bioavailable concentrations.

By improving dosing and exposure characterization, this work advances testing strategies for hydrophobic substances. These findings support more reliable hazard identification and strengthen IVIVE, with implications for regulatory assessments and broader application to difficult-to-test chemicals in environmental toxicology.

1.14.P-Mo089 Development of a non-radiolabelled mixture approach to obtain wastewater primary biodegradation half-lives for hydrocarbons according to the OECD 314B protocol

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Kingdom, (3)Shell Global Solutions International BV, Netherlands, (4)European Fuel Manufacturers Association Division Concawe, Brussel, Belgium

Biodegradation in wastewater treatment plants (WWTP) is the principal removal process for most widespread man-made organic chemicals. As such, estimation of WWTP biodegradation half-lives (HL_{bio}) is critical during the environmental exposure and risk assessment of chemicals. Unlike other environmental substance properties, HL_{bio} cannot be adequately predicted from general physico-chemical properties, such as the octanol-water partitioning coefficient. Instead, HL_{bio} is mainly determined by the presence or absence of rapidly biodegradable sub-structural features (chemotypes). To generate biodegradation data that could be used to develop predictive models, we have selected 68 commercially available hydrocarbons that represent the major chemotypes observed in typical petroleum/hydrocarbon UVCBs (Unknown or Variable composition, Complex reaction products, Biological materials).

To generate realistic WWTP HL_{bio}s, we are using the OECD 314B (activated sludge die-away test) test guideline, which simulates widely-used biological sewage treatment. The OECD 314B TG often requires radiolabeling of the (single) test chemical. However, to generate realistic WWTP HL_{bio}s in a cost-effective manner, we are developing an approach to test mixtures of the 68 commercially available hydrocarbons without radiolabelling.

A non-radiolabeled mixture of 5 representative hydrocarbons (n-decane, propylcyclohexane, 1,3-diethylbenzene, adamantane and diphenylmethane) was used for method development. Technical improvements to the standard protocol were required to 1) ensure stable oxygen concentrations throughout the test, 2) slow down and reduce rapid and substantial abiotic losses (volatilisation and formation of non-extractable residues) of the test chemicals, and 3) eliminate remnant biological activity in the abiotic control vessels.

This presentation shares the method development process and learnings. Once these technical challenges have been resolved, the improved 314B protocol will be applied to the remaining 63 representative hydrocarbons to generate primary biodegradation half-lives for all 68 chemicals. The primary biodegradation half-lives could in theory then be linked to the major chemotypes represented by those 68 chemicals, which would enable better predictive models.

1.14.P-Mo090 Passive Dosing for Identifying Microbial Degradation Products in Heating Oils

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Heating oil stored in tanks is prone to microbial degradation. Quantifying microbial activity in heating oil storage tanks requires significant time and fuel. In this study, we present a rapid and miniaturized system to assess microbial activity in heating oils using passive dosing. Passive dosing avoids the formation of micro emulsions often encountered while handling difficult liquid test chemicals like heating oils. These micro emulsions make it difficult to analytically measure the freely dissolved constituents. To solve this problem, silicone polymer O-rings were loaded with six different heating oils by means of passive dosing, followed by desorption of chemicals into the aqueous test medium to create water-accommodated fractions (WAFs) and additionally buffering losses due to sorption effects. WAFs were analyzed for aromatics, aliphates, alcohols, ester, ether and nitrogen compounds using GC-MS and compared to WAFs prepared from direct dosing experiments. GC analysis revealed a consistent WAF composition across both passive and direct dosing for all tested oils, with minor differences in xylene content. However, the absolute quantities varied considerably across oils. The substance breakdown showed a dominance of monoaromatic hydrocarbons, followed by diaromatics and heterocycles. Microbial activity in one heating oil was determined by measuring CO₂ in the headspace of a closed system. After microbial activity ceased, the aqueous test medium was extracted using silicone O-rings as passive samplers followed by GC-MS analysis to determine microbial degradation products. Except for indanes, all major substance classes were markedly reduced; xylenes were no longer detectable in one replicate. In addition, phenols appeared as intermediates in the degradation of benzenes. Shaking enhanced the release and bioavailability of heating oil components, while a reduced headspace volume allowed for quicker CO₂ detection. These results demonstrate the potential of passive dosing as a rapid, cost-effective method for evaluating the microbial degradability of heating oils.

1.14.P-Mo091 Examining biodegradation of a UVCB using distilled fractions

Paul G. Koster van Groos¹, Gail Bragin¹, Jolanta Zukowski¹, Lesheng Wang¹, Maria Likhogrud¹, Cary

Sutherland¹, Jay Patel¹, Louise Camenzuli² and Thomas Delfosse², (1)ExxonMobil, United States, (2)ExxonMobil, Belgium

Biodegradation is a key process affecting the environmental fate of chemicals. For organic chemicals, biologically mediated degradation can result in CO₂, a natural end-product of oxidation; and this process is commonly used to evaluate biodegradability with standardized tests. However, evaluating degradation solely through CO₂ evolution is challenging for UVCBs (Unknown or Variable composition, Complex reaction products, or Biological materials) because CO₂ does not indicate individual constituents that degrade.

We describe efforts examining the biodegradability of a UVCB substance that contains four main types of constituents observable through GC/MS evaluation. One of the four constituent groups is reasonably soluble, but solubilities of the other three constituent types are suggested to be much lower, which may limit bioavailability and biodegradation rates. To facilitate more detailed investigations, the UVCB substance was distilled into individual fractions generally covering each of the four constituent groups. Results from OECD 301F biodegradation screening of the whole substance and individual fractions are described, together with GC/MS analyses of the same. The GC/MS efforts provided further insights regarding the biodegradation that occurred, and also suggested that careful evaluation of whole substance information may provide valuable insights into the biodegradation of the individual constituent types. Overall, the results demonstrated that constituents degraded at different rates, with degradation of the most water soluble constituents occurring rapidly and completely. The extent to which mass transfer limitations due to solubility constraints limited biodegradation was not determined in this work but warrants further consideration.

1.14.P-Mo092 The Practicality of Adjusting pH to Comply with Pharmaceutical Testing Requirements *Tracey Jayne Goodband, Smithers, United Kingdom*

EMA Environmental Risk Assessment requirement guideline (2024), indicates that tests should be conducted at a stable pH consistent with the most bio-available form of the test chemical (usually the non-dissociated form or the form with the most neutral molecule species). However, what if the required pH is close to or outside the recommended pH values for the test species? How far should this be taken before the performance of the test is potentially compromised? And, how practical is this? This presentation will discuss the measures taken to perform a sediment-water chironomid test to the OECD 218 test guideline whilst also taking into consideration the requirements for pH adjustment. This will include initial considerations, the modifications performed during test set up, how effective these were, control performance, potential issues and how it is possible to modify testing in order to best achieve the ERA requirements whilst still maintaining the integrity of the test.

1.14.P-Mo093 Dietary exposure in ecotoxicological studies with fish - a different route for difficult substances

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Ecotoxicological studies with aquatic species usually consider water as the dominant route of exposure. However, in case of difficult substance properties e.g. high lipophilicity or insoluble character, this induces technical challenges. An option might be to apply those substances via the food to ensure a full uptake. Currently, only the bioaccumulation test regimes provide an option for dietary exposure.

In our study, we aimed to assess potential long-term effects of a polymer on the biological performance of exposed fish. However, within an extensive pre-test program, it was not possible to achieve a constant exposure scenario via the water due to adsorption to surfaces and clogging of the polymer. Thus, it was agreed to choose the dietary application route.

Using experiences from fish metabolism and biomagnification studies, we modified the feed preparation procedures for small pelleted fish food. However, this was still not suitable to be used for larvae feeding as part of an Early life stage toxicity test (OECD 210), which is the usual setup for chronic fish toxicity. Instead, a Juvenile Growth test with rainbow trout, following OECD Guideline 215, did ensure a sufficient fish size already at test start.

Fish feed pellets with radiolabelled polymer at three different concentrations (i.e. 0.01, 0.1 and 1.0 % [w/w]) were produced. A pre-test using fish was applied to ensure feed acceptance. In the main-test, the fish were exposed to the test diet for 4 weeks in a flow-through system. Feed amounts were adapted considering the fish size increase during the study. To ensure a correct balancing of the radiolabelled test item, an extensive filter

system was installed to enable a thorough collection of test item as part of the system drain. In addition to the ecotoxicological assessment of the fish, the fish were prepared by excision of the inner organs after exposure. The separate measurements of radioactivity in fish tissue and intestines provided additional information about a possible intake of the test item by fish.

Finally, the study could successfully be conducted in line with the guideline requirements, confirming that a dietary exposure can also be implemented in ecotox testing.

The poster shows anonymised data and the developed test setup. The different project milestones and respective outcomes are illustrated.

1.14.P-Mo094 Assessing Repellent Effects in Bee Toxicity Studies

Sylviane Gony¹ and **Sophia Buschmann²**, (1)*Eurofins Agrosciences Regulatory Services, France*, (2)*Eurofins Agroscience Services Ecotox GmbH, Germany*

The recent EFSA guidance on pollinator risk assessment (EFSA, 2023) is not yet in force. However, its new approach for evaluating bee risk no longer relies on standard endpoints such as 48h-LD50 (acute toxicity), 10d-LDD50 (chronic oral toxicity), or NOED (larval toxicity). Instead, it focuses on using dose–response curves to determine a substance hazard to bees. In some cases, generating such curves can be challenging due to technical limitations, such as very low toxicity or poor solubility. Among these challenges, the repellent properties of a substance are particularly critical, as they may restrict effective exposure and be misinterpreted as reduced feeding motivation rather than a true avoidance response. Therefore, it is essential to demonstrate that the substance does not cause a sustained change in feeding motivation and that any observed decrease in feeding rate is due to a non-toxic, repellent-driven avoidance effect rather than a toxic mechanism. This demonstration should follow OECD testing guidelines without altering the study protocol in a way that could compromise regulatory validity.

For honeybee studies, this is relatively straightforward: the OECD TG 213 (acute oral toxicity) and TG 245 (chronic oral toxicity) designs (10 bees per cage) allow for choice tests by adding a second feeder—one with contaminated solution and one with uncontaminated sucrose. In contrast, bumblebee testing under OECD TG 247 (acute oral toxicity) uses single housing (one bee per Nicot® queen cage), permitting only one feeder and thus no choice between contaminated and uncontaminated solutions. To address this, a test was conducted with a fungicide formulation that previously showed a strong feeding rate reduction in a rangefinder, aiming to confirm that appetite was not durably affected, and that repellency was likely the cause for the observed reduction—without modifying the OECD TG 247 exposure design.

1.15 PFAS Across the Environment–Human Continuum: From Molecular Mechanisms to Disease-Relevant Outcomes

1.15.T-01 A One Health Perspective on PFAS Hazard: System-Level Immune and Neural Disruption in Ecological and Human Models

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Per- and polyfluoroalkyl substances (PFAS) are globally persistent contaminants increasingly implicated in immune dysfunction and neurotoxicity, yet cross-species, mechanistic evidence integrating these endpoints remains limited. We applied a One Health toxicology framework combining human immune cells, a soil invertebrate model, and neuron-like cells to capture convergent PFAS effects on immune and nervous systems. Primary human PBMCs were exposed to PFOS (3–600 µM) under LPS or PHA stimulation and profiled by cytokine arrays and DIA-proteomics. PFOS did not induce overt cytotoxicity but caused marked, stimulus-specific immunomodulation: IL-6 and TNF-α were significantly suppressed in LPS-activated cells, while PHA-evoked Th1/Th2 and CD28 co-stimulatory pathways were repressed. Across conditions, CD48 was consistently downregulated and BAX upregulated, indicating impaired activation and a shift towards a pro-apoptotic phenotype.

Chronic exposure of *Eisenia fetida* to PFAS-contaminated soils (PFOS up to ~880 µg/kg) produced pronounced physiological disruption. Immune genes (Lysenin, CCF-1) showed non-monotonic regulation, catalase and phenol oxidase activities were significantly depressed, and acetylcholinesterase inhibition correlated with delayed escape responses, evidencing neuromuscular and immune impairment. Reproductive output was strongly reduced in high-PFAS soils, revealing ecologically relevant sub-lethal effects.

In S1 neuroblastoma cells, patch-clamp recordings showed that several emerging PFAS (PFMOPrA, PFMOBA, PDMDA, PFuDA, CH₃-PFO₃-3-6-9-TriDoA) significantly and reversibly decreased GABA-evoked currents after pre-treatment, whereas HFPO-DA consistently enhanced GABA responses without activating receptors per se. Docking analyses supported pore-region engagement for inhibitory congeners, consistent with functional modulation of GABA_A receptors.

Together, these data demonstrate that PFAS disrupt immune and inhibitory neurotransmission through distinct yet functionally convergent pathways across species. This integrated evidence base strengthens mechanistic support for PFAS-induced immunosuppression and neurofunctional alteration and underscores the need for cross-species risk assessment within a One Health perspective.

Disclaimer/Disclosure:

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1.15.T-02 Early-life Hexafluoropropylene Oxide Dimer Acid (HFPO-DA or GenX) Exposure Disturbs Circadian Rhythms via DRP-1-dependent Mitochondrial Dysfunction in *Caenorhabditis elegans*

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Poly- and perfluoroalkyl substances (PFAS) are widespread synthetic chemicals used in numerous consumer and industrial applications. They are globally detected in diverse environmental matrices and are associated with multiple toxic effects. Hexafluoropropylene oxide dimer acid (HFPO-DA, or GenX), an emerging PFAS introduced as a replacement for perfluorooctanoic acid (PFOA), remains insufficiently characterized in terms of its biological impact. Given the central role of circadian rhythms in organismal health, we examined whether HFPO-DA exposure disrupts circadian regulation in the model organism *Caenorhabditis elegans* and investigated the underlying mechanisms. We found that prolonged exposure to HFPO-DA or PFOA at 10 µg/mL disrupts circadian rhythms in *C. elegans*. HFPO-DA-induced circadian defects were accompanied by pronounced mitochondrial fragmentation and reduced mitochondrial membrane potential. We further demonstrated that HFPO-DA-induced mitochondrial damage and reactive oxygen species production depend on the mitochondrial fission regulator dynamin-related protein 1 (DRP-1). Moreover, HFPO-DA exposure exacerbated oxidative stress and reduced heat-stress resistance in a DRP-1-dependent manner. In silico molecular analyses supported these findings by revealing stable interactions between HFPO-DA and DRP-1. Collectively, our results show that long-term early-life exposure to HFPO-DA disrupts circadian rhythms through DRP-1-dependent pathways, impairs mitochondrial integrity, and diminishes stress resistance in *C. elegans*. Given the conserved nature of circadian and mitochondrial regulation, along with the increasing global use of HFPO-DA, our study highlights the urgent need to assess the long-term health risks associated with the emerging PFAS contaminant.

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1.15.T-03 Toxicological effects of the emerging PFAS HFPO-DA in mussels exposed to salinity fluctuations: Implications for Future Coastal Ecosystem Health

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Poly- and perfluoroalkyl substances (PFAS) are highly persistent pollutants of growing environmental concern, particularly in transitional ecosystems. Among them, hexafluoropropylene oxide dimer acid (HFPO-DA, also known as Gen-X) has emerged as a replacement for legacy PFAS, yet its toxicity profile in marine organisms remains poorly characterised. At the same time, climate-driven salinity shifts, resulting from sea-level rise, extreme rainfall, and drought, are expected to act as major co-stressors for estuarine species and to modulate contaminant toxicity. To evaluate the interaction between HFPO-DA and salinity, the mussel *Mytilus galloprovincialis* was chronically exposed (28 days) to environmentally relevant HFPO-DA concentrations (10 and 100 ng/L) under three salinity regimes reflecting present and projected climatic conditions (20, 30, 40).

Metabolic, antioxidant, biotransformation, redox, neurotoxicity and histopathological endpoints were integrated using Principal Coordinates Ordination (PCO) and the Integrated Biological Response (IBRv2) index to characterise whole-organism stress responses. Results demonstrated that salinity is the primary driver of mussel responses, with HFPO-DA acting as a secondary modulator. At low salinity (20), IBR values were highest across treatments, but despite enhanced metabolism and strong activation of antioxidant and biotransformation pathways, no tissue or neurotoxic damage was detected. These findings suggest that osmotic stress primes defence capacity and temporarily masks HFPO-DA toxicity. Conversely, at hypersaline conditions (40), antioxidant and detoxification responses were largely constrained, and HFPO-DA exposure resulted in elevated lipid peroxidation, acetylcholinesterase inhibition, and marked gills and digestive gland lesions, indicating that high salinity intensifies HFPO-DA-induced cellular and tissue damage. At salinity 30, HFPO-DA alone was sufficient to induce a distinct metabolic and histopathological signature, particularly at 100 ng/L. Overall, this study reveals that HFPO-DA toxicity is strongly shaped by salinity, with contrasting biological outcomes according to climate-driven scenarios. These findings highlight the need to incorporate salinity variability into ecological risk assessment of emerging PFAS and reinforce the vulnerability of estuarine ecosystems to combined chemical and climate stressors

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1.15.T-04 Protein-Mediated Modulation of PFOA and PFOS Mitochondrial Toxicity in Zebrafish Embryonic Cells (ZF4)

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Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemicals that accumulate in aquatic organisms and interact strongly with serum proteins, potentially altering their cellular bioavailability and toxicity. Understanding how protein binding influences PFAS-induced mitochondrial dysfunction is critical for refining in vitro hazard models and interpreting bioassay outcomes. This study investigated how protein presence in the medium modulates the mitochondrial toxicity of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in zebrafish embryonic cells (ZF4). Cells were exposed to several concentrations (100–1000 µM) of each compound in complete culture medium (CCM, with serum) or serum-free medium (NSM) for 24 h. Cytotoxicity was evaluated using metabolic (CKK-8) and cell-counting (Trypan Blue) assays, while mitochondrial endpoints were assessed using TMRM (membrane potential), MitoSOX Red (superoxide production), and Seahorse XF metabolic profiling. Intracellular PFAS accumulation was quantified by LC–MS to evaluate protein-mediated uptake differences.

Results revealed a pronounced protein-dependent modulation of PFAS effects as well as distinct mode of action differences between PFOA and PFOS. In serum-free conditions, both compounds but particularly PFOA, induced greater mitochondrial depolarization, elevated superoxide formation, and decreased coupling efficiency and basal respiration compared to the same exposures in the presence of serum. Seahorse profiling showed a shift toward glycolytic ATP production and reduced spare respiratory capacity, consistent with mitochondrial dysfunction, following exposure to PFOA that was enhanced under the serum-free conditions. LC–MS analysis confirmed approximately tenfold higher intracellular PFOA levels in NSM relative to CCM, linking enhanced bioavailability to stronger bioenergetic disruption. PFOS elicited comparatively milder effects under both serum-free and serum-containing conditions, despite its intracellular levels being > tenfold higher. These findings demonstrate that serum proteins substantially attenuate PFAS-induced mitochondrial stress by limiting cellular uptake and bioavailability. The data highlight the importance of medium composition when interpreting in vitro PFAS toxicity and the need to account for protein binding when translating cell-based results to environmental exposure scenarios.

Keywords: PFAS, Zebrafish, Mitotoxicity, Protein binding, ZF4

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1.15.P PFAS Across the Environment–Human Continuum: From Molecular Mechanisms to Disease-Relevant Outcomes

1.15.P-Mo095 Ecotoxicological Effects of Long-Chain PFAS in *Mytilus galloprovincialis*: Evidence Across Multiple Biological Levels

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Long-chain per- and polyfluoroalkyl substances (PFAS) are highly bioaccumulative and persistent in marine environments, posing a significant threat to aquatic organisms. This study investigated the biochemical, physiological, and histopathological effects of 4 long-chain PFAS (PFUnDA, PFDoA, PFTrIDA, and PFTeDA) on the mussel *Mytilus galloprovincialis*. The Integrated Biomarker Response (IBR) index was employed to combine metabolic, oxidative, detoxification, and neurotoxic biomarkers and provide an overall measure of organismal stress, enabling the visualization and comparison of biological responses across treatments and species. The results demonstrated that the longest-chain PFAS (PFTrIDA, PFTeDA) induced pronounced metabolic activation, reflected by enhanced electron transport system activity and respiration rate, accompanied by higher protein and lipid reserves and carbohydrates depletion. These energetic shifts intensified aerobic metabolism and higher energetic costs under chemical stress. The upregulation of antioxidant enzymes and total antioxidant capacity in response to increased lipid peroxidation suggests that the extent of oxidative damage exceeded the efficiency of the antioxidant defense system. Biotransformation enzymes displayed compound-specific modulation, with maximal activation under PFDoA and limited induction under longer-chain PFAS, suggesting constrained detoxification efficiency under high oxidative demand. Moreover, inhibition of acetylcholinesterase across all treatments indicated potential neurotoxic impairment. The histopathological analyses conducted in gills, digestive gland, gonad, kidney, and nervous tissue corroborated the biochemical results. Gills and digestive gland showed higher histopathological indices under longer-chain PFAS exposure, mainly characterized by digestive tubule atrophy, brown cell aggregates in primary tubule epithelia, fibrous tissue formation in connective areas, and enlarged central vessels in gills. No significant differences were observed in gonadal or adipogranular index. Kidney lipofuscin accumulation increased with PFAS chain length, and neural tissues exhibited greater necrosis. Overall, PFAS toxicity was chain length-dependent, with PFTrIDA and PFTeDA identified as the most disruptive compounds, eliciting oxidative, metabolic, and neurotoxic imbalances. These findings provide mechanistic insights into PFAS-induced stress in bivalves and support improved ecological risk assessment of PFAS.

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1.15.P-Mo096 Effect of Equimolar PFAS Mixtures on *Eisenia fetida* Immune System

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Per- and polyfluoroalkyl substances (PFAS) are persistent soil contaminants, yet their effects on soil invertebrates remain comparatively understudied relative to aquatic species and human health models. Our laboratory has recently characterised the toxicity of individual PFAS congeners in *Eisenia fetida* following OECD Test 207 guidelines. However, environmental PFAS contamination rarely consists of single compounds; instead, it encompasses complex mixtures of legacy long-chain acids and emerging short-chain ether analogues. This raises a key toxicological question: do equimolar mixtures reproduce, attenuate, or amplify the sublethal responses observed for single compounds, or do they elicit qualitatively distinct biological outcomes?

To begin addressing this knowledge gap, we investigated the immune- and stress-related effects of an equimolar mixture composed of five PFAS: the long-chain legacy acid perfluorooctanoic acid (PFOA, C8), the short-chain perfluorobutanoic acid (PFBA, C4), and three emerging ether-PFAS—HFPO-DA (GenX, C6), PFMOBA (C5), and PFMOPrA (C4). Adult *E. fetida* were exposed on filter paper for 72 h to four nominal concentrations (0.6, 4.2, 31 and 229 µM). To disentangle mixture interactions, each PFAS was also tested individually at concentrations matching its proportion in the mixture, enabling direct comparison between combined and single-congener exposures.

Coelomic fluid was collected and separated into cellular and acellular fractions. In the supernatant, biochemical endpoints were quantified, including acetylcholinesterase (AChE), phenoloxidase (PO), and catalase (CAT), representing neurotoxicity, immunomodulation, and oxidative stress, respectively. The cellular pellet was used for amoebocyte morphometry, micronucleus scoring, and evaluation of cytological and genotoxic alterations. Furthermore, relative expression of CCF-1 and Lysenin, two genes involved in immune defence and stress responses, was assessed.

By integrating biochemical, cytological, and molecular biomarkers, this study aims to establish a comprehensive framework for interpreting the adverse outcome patterns potentially elicited by equimolar PFAS mixtures compared to their individual components. At this stage, the dataset allows us to define a robust experimental platform for mixture toxicity assessment; detailed interpretation of biological trends will require completion of ongoing analyses.

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1.15.P-Mo097 Immunohistochemical Mapping of Neurotransmitter Systems in *Eisenia fetida* Exposed to PFAS

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants whose sub-lethal effects on terrestrial invertebrates require sensitive mechanistic tools. *Eisenia fetida* is a relevant terrestrial model for neuroecotoxicology, yet methods capable of detecting PFAS-driven alterations in specific neurotransmitter systems remain limited.

We developed and applied a neurochemical histology workflow to evaluate the effects of two PFAS congeners—perfluorooctanoic acid (PFOA) and HFPO-DA (GenX)—on cholinergic, GABAergic, and serotonergic signalling in *E. fetida*. Adult worms were exposed to 0.06 µM and 229 µM of each compound, after which fixed and embedded organisms were serially sectioned for targeted immunohistochemical analyses. Cholinergic activity was assessed using Karnovsky's histochemical method for acetylcholinesterase (AChE). GABAergic modulation was examined using anti-GABA immunofluorescence, enabling quantification of GABA immunoreactivity in the circular muscle layer and peripheral neural structures. To expand the neurochemical panel, serotonergic innervation was characterised through anti-serotonin (5-HT) immunolabelling, allowing detection of serotonergic fibres and mapping of their spatial distribution under PFAS exposure. All imaging procedures were standardised for semi-quantitative comparison across treatments. Preliminary observations revealed detectable alterations in AChE, GABA, and 5-HT immunoreactivity in PFAS-exposed specimens relative to controls, confirming the sensitivity of this multiparametric histological platform in capturing PFAS-induced neurochemical shifts.

This methodological framework provides an expanded and reproducible approach to investigate neurotransmitter-level endpoints in soil invertebrates, offering finer mechanistic resolution and complementing traditional biochemical or behavioural assays in PFAS neuroecotoxicology.

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1.15.P-Mo098 Exploring Native Fungal Communities for PFAS Tolerance in Contaminated Soils

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental pollutants that pose significant ecological and human health risks due to their chemical stability and resistance to degradation. Among them, perfluorooctane sulfonate (PFOS) is the most prominent compound in our study area, a drill site with long term history of legacy PFAS contamination in Trelleborg, Sweden.

The goal of this study was to determine whether fungi native to PFOS-contaminated soils harbor adaptive traits enabling them to tolerate or even utilize PFAS compounds. We isolate and characterize fungal strains from the most contaminated soil sample (910 ppb sum of PFAS4 -sensu EFSA) and assess their growth on minimal PDA medium enriched with increasing concentrations of PFOS (10, 100, and 1000 mg/L).

Among the isolates, *Cladosporium cladosporioides* displays robust and consistent growth at all tested concentrations, suggesting a high level of tolerance to this contaminant. This observation aligns with previous literature reporting the genus's capacity to manage a variety of xenobiotic compounds. Based on these results, we select *Cladosporium cladosporioides* for preliminary chemical analyses on the culture medium. To evaluate the effect of fungal activity on PFOS concentrations, we perform LC-MS/MS analyses on culture media and fungal biomass after 30 days of growth. These analyses show a higher concentration of PFOS the medium closest to the fungal mycelium with the appearance of PFHxS, a shorter-chain PFAS, absent in control samples with no fungal growth. Meanwhile, only low levels of PFOS accumulate within the fungal biomass, which suggests that the fungus does not significantly internalize the contaminant but may instead be contributing to its transformation externally. These observations suggest a possible chain-shortening conversion of PFOS, supporting the hypothesis that fungi isolated from PFOS-contaminated environments may evolve adaptive or metabolic mechanisms to tolerate and potentially biotransform PFAS.

Our findings highlight the resilience and functional potential of native fungal communities in PFAS-polluted soils. Ongoing optimization of analytical protocols and molecular analysis will help confirm the identity and ecological relevance of the isolated strains, laying the groundwork for future applications of native fungi in sustainable bioremediation strategies targeting PFAS-contaminated environments.

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1.15.P-Mo099 Neurofunctional and Transcriptomic Responses to the Hexafluoropropylene Oxide Analogues HFPO-DA and HFPO-TeA

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Among the emerging ether-perfluoro analogues, hexafluoropropylene oxide dimer acid (HFPO-DA) and tetramer acid (HFPO-TeA) have been introduced as replacements for legacy PFAS, yet their potential neurotoxic properties remain poorly characterized.

In this study, we investigated their effects on neuronal homeostasis using S1 neuroblastoma cells with GABAergic features. We combined transcriptomic profiling, electrophysiological recordings of GABAA currents, cytotoxicity assays, and molecular docking.

Cytotoxicity testing revealed distinct profiles: in standard submarine culture conditions, HFPO-DA caused no changes in cell viability, whereas HFPO-TeA reduced viability at millimolar concentrations. These findings point to a lower cellular tolerance for HFPO-TeA.

Patch-clamp recordings showed that HFPO-DA (10 μ M) significantly and reversibly enhanced GABA-evoked currents without activating receptors on its own, suggesting a modulatory action at non-orthosteric sites. In contrast, HFPO-TeA, tested at reduced concentrations to preserve membrane integrity (0.5 μ M), did not affect GABA responses, indicating minimal acute interaction with GABAA receptor function.

Molecular docking revealed peripheral, non-occlusive poses for HFPO-DA, consistent with its lack of pore-blocking activity, whereas HFPO-TeA showed deep pore insertion in the closed state but retracted toward the channel exit in other states, a pattern that did not match its absence of current modulation.

Transcriptomic analysis (1 or 10 μ M) further revealed some compound-specific signatures. Although HFPO-DA and -TeA shared several enriched KEGG and GO pathways related to mitochondrial respiration, oxidative phosphorylation, ribosome biogenesis and proteostasis, -TeA elicits a far stronger, coherent and dose-dependent

activation of this program. It also triggered broader dysregulation of oxidative-stress- and mitochondrial-related pathways, including MAPK, apoptosis, and p53 signaling.

These findings reveal distinct neurotoxicological potential for HFPO-DA and HFPO-TeA. HFPO-DA acts as a mild and reversible modulator of GABAergic signaling with limited cytotoxic effects, whereas HFPO-TeA shows reduced cellular tolerance and induces transcriptional responses consistent with cellular stress. These results highlight divergent mechanisms for structurally related HFPO analogues and underscore the need for further studies to evaluate their long-term effects on neuronal systems.

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1.15.P-Mo101 In Vitro Structure–Activity Relationships and Relative Potency Factors of PFAS: Integrating Hepato- and Immunotoxicity Endpoints for Improved Risk Assessment

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Background: Per- and polyfluoroalkyl substances (PFAS) constitute a chemically diverse group of persistent pollutants for which toxicological data remain limited, particularly for emerging short-chain and fluorotelomer-based compounds. To support risk assessment of data-poor PFAS, we systematically investigated the structure–activity relationship (SAR) of thirteen PFAS, including perfluoroalkyl acids (PFCAs), perfluorosulfonic acids (PFSAs), and fluorotelomer alcohols (FTOHs), in human hepatocytes (HepG2) and immune cells (THP-1 monocytes/macrophages).

Methods and results: Across cytotoxicity assays and intracellular reactive oxygen species (ROS) assays, PFAS toxicity increased with carbon-chain length up to 8–10 carbons, while functional headgroups strongly influenced potency. PFCAs and PFSAs showed pronounced concentration-dependent cytotoxicity and ROS induction, whereas FTOHs were minimally toxic in HepG2 cells but exhibited marked immunotoxicity, underscoring clear cell-type specificity. Mechanistic analyses revealed that mitochondrial disruption acts as an early molecular initiating event, triggering ROS-generating cascades involving lysosomes and peroxisomes. In THP-1 macrophages, several PFAS altered cytokine responses by modulating TLR4-mediated signaling, supporting immunotoxicity as a particularly sensitive endpoint.

Significance: Using cytotoxicity- and ROS-based endpoints, we derived in vitro Relative Potency Factors (RPFs), revealing consistent potency rankings across PFAS subclasses. These RPFs provide a quantitative basis for grouping, prioritization, and read-across, demonstrating the feasibility of SAR-driven approaches for improving the risk assessment of both legacy and emerging PFAS. Collectively, our findings show that PFAS toxicity is structure-dependent, endpoint-specific, and cell-type-specific, supporting the integration of NAM-based SAR frameworks into regulatory evaluation.

1.15.P-Mo102 Enhancing Access to Human Biomonitoring Data in Europe: The Development and Utility of the European HBM Dashboard

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HBM data serves as a crucial measure of internal concentration levels of exposure biomarkers found in human matrices, including urine and blood. As part of the Human Biomonitoring for Europe project (HBM4EU), the Flemish Institute for Technological Research (VITO) developed a comprehensive human biomonitoring (HBM) dashboard, launched March, 2021. Currently including data from over 100 studies covering 25 European countries. This dashboard continues to be actively maintained and updated under the auspices of the Partnership for Assessment of Chemical Risks (PARC) and aims to broaden access to HBM data by providing an intuitive platform for visualizing summary statistics derived from HBM datasets, fostering widespread community engagement. Users can explore exposure levels and trends in chemical exposure across European populations, with the ability to compare these levels against established health-based guidance values.

The dashboard ensures data consistency and reliability through standardized data collection methodologies, including predefined data templates, codebooks, data validation, derived variables and summary statistics tools (<https://hbm.vito.be/tools>) The dashboard presents key statistics i.e. (P05, P10, P25, P50, P75, P90, P95). An array of filters enables customized data exploration by criteria like sampling year, country, or target population.

Users can visualize summary statistics for specific stratifications, facilitating subgroup comparisons like sex or educational level.

The European HBM Dashboard represents a significant advancement in promoting transparency and accessibility in human biomonitoring. Its user-friendly interface and robust data visualization tools, make it a valuable resource for policymakers, researchers, and stakeholders in public health and environmental risk assessment. The dashboard, publicly accessible online at: <https://hbm.vito.be/eu-hbm-dashboard>, promotes informed decision-making and fosters engagement with HBM data.

1.15.P-Mo103 Extracellular Vesicles as Novel Indicators of PFAS-Induced Molecular Responses in Zebrafish Embryos

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Extracellular vesicles (EVs) are nanosized, lipid bilayered particles released by most cell types. They carry proteins, lipids, and nucleic acids from their parent cells, mediating intercellular communication. EVs can reflect exposure- or stress-induced alterations in cellular function, offering a potential insight into molecular responses to contaminants.

Zebrafish (*Danio rerio*) embryos are an established model for studying vertebrate development and toxicological mechanisms. Despite growing interest in extracellular vesicles, studies in zebrafish remain scarce, with only a few describing EVs under baseline conditions and none addressing exposure to environmental contaminants. This project aims to fill this gap by investigating how per- and polyfluoroalkyl substances (PFAS) modulate EV production and cargo composition in developing zebrafish.

PFAS are persistent synthetic chemicals widely used in industrial and consumer products. Owing to their extreme stability, they accumulate in the environment and organisms, where they can interfere with lipid metabolism, immune function, and cellular signaling.

The project aims to establish a workflow for isolating and characterizing extracellular vesicles from zebrafish embryos and to determine how PFAS exposure alters their size, abundance, and molecular composition. Embryos will be exposed to increasing concentrations of PFAS, and embryonic development will be assessed for potential morphological and physiological effects. EVs will be isolated and their morphology visualized by cryo-electron microscopy, while particle size and concentration will be measured by multi-angle dynamic light scattering, and small RNA profiling will be applied to assess PFAS-induced changes in EV cargo.

This study will provide in vivo insights into contaminant-induced modulation of EVs during vertebrate development. The outcomes are also expected to advance understanding of PFAS-mediated molecular communication and evaluate the potential of EVs as sensitive, mechanistically relevant biomarkers in aquatic toxicology.

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1.15.P-Mo104 Placental ASCL2 Epimutations Link Prenatal Perfluorooctanoic Acid Exposure to Gestational Diabetes Mellitus

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Prenatal exposure to perfluorooctanoic acid (PFOA) is associated with an increased risk of gestational diabetes mellitus (GDM). The Achaete-scute family bHLH transcription factor 2 (ASCL2) plays a critical role in trophoblast differentiation during placentation; however, the mediating role of ASCL2 in disrupted glucose homeostasis following gestational PFOA exposure remains unclear. This study investigated whether PFOA-induced placental epimutations of ASCL2 contribute to GDM pathogenesis using a mouse model and a human birth cohort. Pregnant C57BL/6N mice were exposed to PFOA from embryonic day (E) 6.5 to E15.5, and placentas were collected at E16.5. Human placental specimens were obtained from participants in a Korean birth cohort. Placental mRNA expression levels of *Ascl2* and ASCL2 were assessed by reverse transcription-quantitative PCR (RT-qPCR). DNA methylation changes in the *Ascl2* differentially methylated region (DMR)

and the ASCL2 5'-end region were determined by bisulfite amplicon sequencing (BSAS) in mouse and human placental samples, respectively. RNA sequencing was conducted to profile transcriptomic programming in Ascl2- or ASCL2-upregulated placentas. Prenatal PFOA-exposed mice exhibited elevated maternal blood glucose levels and increased placental Ascl2 expression. In the human cohort, elevated placental ASCL2 expression was notably observed in GDM patients with significantly high maternal serum PFOA levels. Distinct methylation-expression relationships were found between species: positive correlations between Ascl2 expression and CpG methylation in the mouse Ascl2 DMR, whereas inverse correlations were observed in the human ASCL2 5'-end region. Transcriptomic profiling revealed distinct pathway enrichments between species: PFOA-exposed mouse placentas with elevated Ascl2 showed prominent enrichment of oxidative phosphorylation and cell proliferation pathways. In contrast, GDM human placentas with elevated ASCL2 exhibited glycolysis, mTORC1 signaling, and hormone response pathways, reflecting metabolic reprogramming characteristic of diabetic pregnancy. Our findings demonstrate that PFOA-induced placental ASCL2 epimutations are associated with maternal hyperglycemia in mice and GDM in humans. These results highlight critical species differences in placental responses to environmental exposures, and suggest that site-specific ASCL2 DNA methylation could serve as an epigenotoxic biomarker for detecting environmentally-induced GDM.

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1.15.P-Mo105 Resolving The Unknown Toxicity of Novel Per- And Polyfluoroalkyl Substances (PFAS): ADONA, Gen-X and PFOSA

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The global production and widespread use of per- and polyfluoroalkyl substances (PFAS) have resulted in their continuous release into the environment, increasing their concentrations and generating ecotoxicological concerns. This scenario has driven the regulation of legacy PFAS and the subsequent development of new compounds by industry. Related toxicological research has thus largely centered on PFOS and PFOA, leaving other long-chain PFAS, such as the PFOS precursor PFOSA, and new-generation ones like ADONA and GenX less explored.

This study aims to evaluate the toxicological effects of these three under-studied PFAS through a battery of bioassays addressing different levels of biological complexity to clarify their potential environmental and human health impacts. The approach involves assessing effects on sea urchin embryo development, coelomocyte viability in polychaetes, marine bacteria bioluminescence, and human endocrine activity.

Results indicate that ADONA and GenX did not exert environmental impact at the tested endpoints. PFOSA affected sea urchin embryo development at concentrations above 5 mg/L, reduced coelomocyte viability at levels higher than 50 mg/L, and impaired bacterial bioluminescence starting at 5 mg/L, with significant effects at 50 mg/L. Regarding human-related endpoints, ADONA and GenX increased basal estrogenic activity under co-exposure with estradiol (50 ng/L) at concentrations above 50 mg/L, without affecting androgenic or thyroid receptors. PFOSA induced anti-estrogenic activity under estradiol co-exposure and showed anti-androgenic effects at concentrations above 50 mg/L, without influencing the thyroid receptor.

Overall, the findings provide the first reliable toxicological characterization of PFOSA, ADONA and GenX, indicating a toxicity ranking of PFOSA > GenX > ADONA. The multi-endpoint approach used in the present study generates mechanism-oriented evidence that helps bridge molecular actions with organism-level responses, as these pollutants disrupt vital functions. In addition, it provides an integrated overview of the impact of these persistent pollutants from a One Health perspective.

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1.15.P-Mo106 Sniffing out PFOS: Mass spectrometry imaging for tissue-specific effects of contaminants
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Mass spectrometry imaging (MSI) is an exciting emerging technology that adds spatial information to mass spectrometry analysis, allowing mapping of both contaminants and endogenous biomolecules in the tissue. Where traditional mass spectrometry methods involves tissue homogenization, MSI preserves tissue heterogeneity and it is possible to look at extremely small tissues or tissue regions without contamination from the surroundings.

Here we utilized a representative of true frogs, the cosmopolitan tadpole model American bullfrog (*Rana* [Lithobates] catesbeiana), and MSI to identify hotspots of PFOS accumulation and look for associated tissue-specific effects in lipid composition. Premetamorphic tadpoles were exposed to PFOS (0, 10 and 100 µg/L) for 48 h. Tadpole heads were sampled for MSI and analyzed on a Bruker timsTOF fleX-MALDI-2

The results showed the tissue localization of PFOS – which notably localized to a specific neuroendocrine gland in the brain and to the olfactory epithelium. These same tissues exhibited a depletion in glycerophospholipids, with phosphatidylethanolamines (PEs) and phosphatidylglycerol (PGs) comprising the greatest number of PFOS-affected lipids. In the pineal gland, PFOS also induced depletion of the lipid PA (38:3); which has been shown to be important for brain development in mice.

The study exemplifies how MSI advances our analytical toolbox to support unbiased screening of contaminant distribution and their localized effects in biological compartments.

1.16.P The Extended Chemical Defensome: Novel Insights Into the Mechanisms of Defense Allowing Species to Cope With Chemical Pollution

1.16.P-Th001 Introducing the extended chemical defensome to emphasize mechanisms of defense as key research avenues to tackle priority questions in environmental toxicology

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In response to chemical exposure, organisms use specific regions of their genome coding for different defense mechanisms—this collection of genes is termed the “chemical defensome”. Specifically, genes associated with efflux transporters, transcription factors, antioxidant systems, and biotransformation pathways, among others, are expressed to reduce toxicity. These sub-individual processes are, for the most part, widely conserved across taxa and play a critical role in enabling organisms to cope with polluted environments. Additionally, behavioral responses—particularly spatial avoidance—should be recognized as an individual-level defense mechanism and incorporated into an extended chemical defensome framework. We therefore propose that expanding and reinforcing the concept of the chemical defensome beyond traditional studies at the genome level, as well as developing strategies to synthesize existing data, offers a valuable opportunity to link gene composition to physiological and behavioral responses, thereby addressing key research needs in environmental toxicology. These include: estimating the impact of chemical mixtures across different exposure scenarios, identifying the main drivers of intra- and interspecific sensitivity to pollution, and assessing large-scale ecological processes, such as biodiversity losses, in polluted habitats in a more integrated manner. In ecotoxicology and environmental risk assessment (ERA), understanding not only how chemical pollutants exert toxicity but also how organisms counteract these effects is essential. Thus, investigating chemical-induced shifts in defense mechanisms and strategically incorporating these data into ERA frameworks could improve predictions of adverse outcomes at higher levels of biological organization and inform more effective chemical management and regulatory strategies.

1.16.P-Th002 Microbial Components of the Host's Chemical Defensome

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Aquatic ecosystems are continually burdened with cocktails of micropollutants, including persistent organic compounds, pesticides, pharmaceutically active substances, and endocrine-disrupting chemicals, posing a hidden threat to fish health. Although laboratory studies have demonstrated the biochemical and metabolomic effects of these contaminants, their influence on the gut microbiome of wild fish, which is critical for immunity, metabolism, and overall health, remains poorly understood. This study evaluated the impact of wastewater treatment plant (WWTP) effluent on the intestinal microbiome of wild brown trout (*Salmo trutta* m. *fario* L.). Fish restocked downstream of an STP were compared with an upstream control group. Trout intestines were sampled after 6, 14, and 24 weeks. DNA extracted from intestinal contents was sequenced for 16S rRNA amplicons, clustered into zero-radius OTUs, and assigned taxonomy using Silva132. Taxonomic composition, microbial diversity, and functional potential were assessed. Upstream fish had a stable microbiome dominated by Firmicutes (249%), Actinobacteriota (224%), and Proteobacteria (222%), whereas downstream fish exhibited a variable microbiome dominated by Chlamydiota and enriched in *Ca. Rhabdochlamydia*, *Ca. Mesochlamydia*, and *Microcystis*, reflecting chemical contamination and potential toxic cyanobacterial influence. Downstream microbiomes showed higher diversity and unevenness, with functional shifts toward taxa adapted to antibiotics, biocides, and xenobiotics, including enhanced xenobiotic degradation, membrane/lipid remodelling, and tryptophan metabolism. The integration of microbiome, functional, and physiological data suggests that WWTP effluent exerts long-term selective pressure on brown trout, restructuring microbial communities and favouring pollutant-adapted, antibiotic-resistant, and opportunistic taxa. These microbiomes may serve as reservoirs of resistance genes and catabolic functions, creating an environment-host interface that facilitates the spread of adaptive traits through the aquatic food web. Such an adapted microbiome may potentially represent microbial components of the host's chemical defensome.

Disclaimer/Disclosure:

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1.16.P-Th003 Sex-linked Insecticide Selectivity in Mason Bees (*Osmia* spp.) is Driven by Differences in Cytochrome P450 Expression Profiles

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Pollinator safety is a critical area for the evaluation and assessment of pesticide safety. Recently, attention has been focused on solitary bees as important group of pollinators, with *Osmia* spp. suggested as suitable laboratory toxicity testing species because of their availability and ease of handling. However, *Osmia* spp. and their physiology remain poorly understood in comparison to the honey bee *Apis mellifera* where decades of research have contributed to a solid knowledge of its physiology and potential determinants of pesticide sensitivity.

Recent studies have reported sex-linked insecticide sensitivity differences in certain *Osmia* species for which the mechanistic understanding remains limited. To address this knowledge gap, we investigated functional capacity and sex-linked differences in expression of cytochrome P450 monooxygenase genes, focusing on the CYP9 and CYP6AQ subfamilies commonly implicated in insecticide metabolism. Functionally recombinant expression of CYP9BU1 and CYP9BU2 of three *Osmia* species confirmed their detoxification capacity in vitro. qPCR revealed pronounced sex-biased transcription: Especially *O. lignaria* females exhibited consistently lower transcript abundance of CYP9 and CYP6AQ subfamilies, with reductions reaching a maximum of 10-fold compared to male counterparts, suggesting reduced detoxification capacity potentially linked to higher sensitivity towards certain insecticides. Subsequent RNAseq analyses were conducted to compare sex-specific transcriptomic profiles of a broader set of detoxification genes across *O. cornifrons*, *O. lignaria*, and *O. bicornis*. Such studies help to elucidate the physiology and sensitivity determinants of *Osmia* species used in toxicity testing, which in turn can help to inform the evaluation of toxicity data derived from such studies and how to integrate such data meaningfully in the evaluation of pesticides.

1.16.P-Th004 Persistent Organic Pollutants in the Barents Sea Marine Mammals: Habitat, Diet, and Biotransformation

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Persistent organic pollutants (POPs) are human-made chemicals that organisms struggle to metabolize, and are thus ubiquitous in the environment as they persist and accumulate in animal tissues. Due to their traits, this particularly affects marine mammals: e.g., they have blubber, a layer of fat used for thermoregulation, in which POPs accumulate; they also usually occupy a high position in the food chain, which makes them susceptible to biomagnification. These animals are imperilled by POPs, and their health has been shown to deteriorate with increasing exposures, eventually leading to higher sickness and mortality rates. Organisms do have mechanisms to protect them from xenobiotics, the so-called chemical defensesome, despite its limited effectiveness against POPs. Usually lipophilic in nature, xenobiotics go through a series of oxidation and conjugation reactions that make them more hydrophilic, and thus easier to excrete from the organism. Here we assess the extent of POP accumulation in the marine mammals of the Barents Sea, using samples of blubber from 14 different species. Hierarchical clustering analysis of the samples grouped them by taxonomy, and the total amount of POPs in each taxonomic group followed a pattern of toothed whales > polar bears > walruses > seals > baleen whales. These results were cross-referenced with existing habitat and diet information for each species, and while these factors helped explain the found POP levels, they did not describe the whole picture. This indicates that additional factors could be vital determinants of pollutant accumulation. Thus, we looked into species-specific variations in biotransformation capacity - we quantified the number of genes in 14 subfamilies involved in crucial biotransformation reactions. Numbers of genes found for each subfamily followed similar trends in each taxonomic group, with polar bears > walruses > true seals > baleen whales > toothed whales. All these results combined made it evident that despite their prominent role, the chemical defensesome repertoire does not determine POP accumulation alone. To conclude, in this work we show how the interplay between environment and biotransformation capacity ultimately determines the differential accumulation of toxic compounds in the blubber, using the marine mammals of the Barents Sea as a case study.

Disclaimer/Disclosure:

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Track 2: Ecotoxicology Becomes Stress Ecology: From Populations to Ecosystems and Landscapes

2.01 Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment

2.01.T-01 Multiomics insights into wild flora molecular adaptation in the Chernobyl Exclusion Zone *Polina Volkova, Independent Researcher, Belgium*

Omic technologies are transforming our ability to understand the long-term consequences of low-dose chronic radiation exposure by moving beyond survival and morphology towards system-level changes in transcripts, proteins, and metabolites. By integrating field-based genomics, transcriptomics, proteomics and metabolomics, these approaches reveal fine-tuning of plant metabolism, signalling, and genome regulation that would remain invisible with traditional endpoints alone.

Comprehensive research on four plant species growing in the Chernobyl Exclusion Zone (*Trifolium repens*,

Achillea millefolium, *Vicia cracca*, and *Chelidonium majus*) using transcriptomics, non-targeted proteomics, and non-targeted metabolomics revealed that chronic radiation exposure drives nitrogen redistribution into stress-related metabolites and secondary compounds with antioxidant and defence functions. Moreover, altered TCA and glyoxylate cycles, suppression of some photosynthetic functions, accumulation of reactive oxygen species (ROS)-scavenging and phenolic metabolites, and signatures of biotin and redox imbalance, point to a tight rewiring of carbon metabolism and ROS homeostasis. Although species-specific reactions were revealed, enrichment of general abiotic-stress response networks, modulation of ROS-related systems (thioredoxin, glutathione), changes in photosynthesis-related genes, and deregulation of histones, heat-shock proteins, and transposable elements were core responses to chronic radiation. Hormone and signalling pathways, particularly abscisic acid-, auxin- and gibberellin-responsive genes, also appear as important integrators of the chronic radiation response, with little or no sustained induction of canonical DNA-repair genes. Together, these omics-based studies provide a more mechanistic, pathway-centred understanding of how plants cope with chronic low-dose radiation exposure and important insights into the adverse outcome pathways specific to ionising radiation in plants. Within the International Commission on Radiological Protection (ICRP) framework for environmental radiation protection, such mechanistic evidence can help refine endpoints for Reference Animals and Plants, supporting the selection of sensitive biomarkers and life stages.

2.01.T-02 Non-target terrestrial plant risk assessment: The implications for weed control in the EU

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In 2024, the EU Commission mandated EFSA to update the risk assessment methodology for the assessment of active substances and plant protection products (PPPs) with respect to their potential effects on non-target arthropods other than bees, in-soil organisms and non-target terrestrial plants (NTTPs) outside of the treatment area. This process will require revision of the Specific Protection Goals and the associated Terrestrial Guidance Document that currently defines the risk assessment scheme for each organism class. EFSA has published a Scientific Opinion addressing the state of the science on the risk assessment of PPPs for NTTPs in 2014. This Opinion raises questions relating to risk assessment for NTTPs, including the suitability of endpoints for vegetative vigour on current test species to address potential effects on other plant species and effects on plant reproduction. In addition, it proposes increases in safety factors to address the uncertainty around these questions. These changes in risk assessment are predicted to limit the ability to register PPPs in the EU, increasing the need for higher-tier risk assessment solutions, such as ecological and landscape level modelling, and ultimately change the availability of PPPs to growers. Since 2014, the SETAC Plant Interest Group held two workshops, which lead to the initiation of research projects to investigate the issues of species sensitivity and plant reproduction. In addition, CropLife Europe has completed impact analyses to identify the likely consequences of proposed changes on the availability of PPPs to growers in the EU. Specifically, in this presentation, we will present findings related to:

- (a) the comparative sensitivity of wild and crop species
- (b) the comparative sensitivity of reproductive and vegetative endpoints
- (c) the impact of increased safety factors on the availability of herbicides in the EU
- (d) the consequences of reduced herbicide availability on weed control methods used by growers
- (e) the impact of these changes on broader socio-economic and environmental factors such as food production, farm costs and soil health.

The issues outlined in this presentation highlight that any future changes to PPP risk assessment will be accompanied by socio-economic trade-offs that need to be considered in light of the wider needs of growers and food production in the EU.

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2.01.T-03 Overspray Exposure of Aquatic Plants: New Experimental Evidence and Implications for Risk Assessment

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Recent EU regulatory reviews, e.g., the glyphosate renewal, have explicitly identified a gap in current aquatic plant risk assessment, as direct foliar exposure scenarios are not yet considered. Although spray drift can result

in direct deposition of plant protection products on floating or emergent aquatic macrophytes, aquatic risk assessments still focus exclusively on exposure via the water phase. Conversely, non-target terrestrial plant (NTTP) assessments address foliar overspray but provide no clear evidence of their protectiveness for aquatic species facing comparable exposure routes. To address this regulatory gap, we conducted two dedicated overspray experiments with *Lemna minor* and *Glyceria maxima* using glyphosate.

For *L. minor*, foliar overspray caused substantially stronger effects than exposure via the growth medium. A risk assessment case study showed that while NTTP endpoints may appear protective at first glance, differing assessment factors and trigger values between terrestrial and aquatic frameworks lead to contrasting regulatory conclusions. For *G. maxima*, overspray resulted in strong growth inhibition and lethality at higher doses, whereas effects from exposure via the growth-medium were minimal, preventing derivation of EC50 values for that route.

Together, these findings demonstrate that foliar exposure represents a relevant and potentially critical risk pathway for aquatic macrophytes that is insufficiently covered by current regulatory approaches. The results highlight the need to re-evaluate the applicability of NTTP assessments for aquatic plants and to reconsider assessment factors resp. trigger values for this intermediate exposure scenario. Additional experimental data across aquatic and semiaquatic species will be essential to draw a robust conclusion on the risk assessment.

2.01.T-04 Aquatic Macrophyte Test Outdoors: Method to Derive a Species Sensitivity Distribution for Time-Variable Exposure

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Uncertainty regarding inter-species variation in sensitivity is reduced by testing multiple species. Towards this aim, 10 aquatic macrophyte species were simultaneously exposed to a herbicide in outdoor tanks for 14 days. Total Shoot Length, Fresh Weight (FW) and Dry Weight (DW) was quantified as in OECD Guideline 239. Growth Rate and Yield of control and treated plants were compared to calculate x% inhibitions for each peak concentration. These were used to derive EC50 values, a Species Sensitivity Distribution (SSD), and an HC5. The method will be presented as an example for potential use in higher-tier risk assessment of Plant Protection Products. The study was in July 2025 in Germany. Tanks were 5 m by 1 m wide. Test species were *Heteranthera zosterifolia*, *Egeria densa*, *Ceratophyllum demersum*, *Myriophyllum spicatum*, *Ranunculus aquatilis*, *Nasturtium officinale*, *Veronica beccabunga*, *Callitriche palustris*, *Mentha aquatica*, and *Lemna gibba*. Replicates per species followed OECD 239. There was one tank per concentration, each tank containing all species. Analysed peak concentrations were 0 (control), 0.0805, 0.238, 0.925, 2.50 and 9.17 µg herbicide/L in Smart and Barko medium. The test substance was dosed into each tank and the medium stirred. To elucidate time-variable exposure-profiles samples were analysed at eight time-points. According to OECD 239 a 2x increase in control fresh weight is sufficient to detect effects. The factor of control fresh weight increase ranged from 3.5 (*M. aquatica*) to 19.2 (*N. officinale*). The DT50 values ranged from 2.98 to 4.76 days. Based on inhibition of Growth Rate, *C. palustris* (FW EC50 0.537 µg/L) and *N. officinale* (FW EC50 0.973 µg/L) were most sensitive and *E. densa* (DW EC50 2.84 µg/L) least sensitive. The HC5 was 0.698 µg/L. The method was essentially the transfer of OECD Guideline 239 to outdoors, for multiple species. The method was valid in terms of good control growth and sensitive in terms of the organism response. In this example the range of sensitivities was relatively narrow, thereby reducing uncertainty with respect to inter-species variation. This method derives an HC5 - for a time-variable exposure - which could be used in higher-tier risk assessments described in EFSA Aquatic Guidance Document (2013).

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2.01.T-05 The Prevalence and Acceptability of Mesocosm Studies Submitted for Macrophytes in Pesticide Risk Assessment

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Mesocosms can be used in higher tier aquatic risk assessments to assess the impact of Plant Protection Products (PPPs) on macrophytes. However, it is unclear whether these expensive and time consuming higher tier studies influence regulatory outcomes. This platform presentation will highlight common shortcomings in the experimental design of mesocosm studies, with the aim of maximising the regulatory value of future mesocosm studies. Fourteen mesocosm studies, which have been submitted for the regulatory risk assessments for macrophytes in the EU or GB, were identified and reviewed. Results show that only five of the 14 mesocosm studies were deemed acceptable by the regulatory authorities, suggesting that mesocosm studies are not currently being used to their full potential. Issues with the submitted studies include not following a realistic PPP exposure profile (including incorrect dose timings and dilutions), only using one macrophyte morphology, not leaving enough time for the macrophytes to establish and a lack of replicates which increases variability within treatments. *Glyceria maxima* and *Myriophyllum spicatum* were frequently the most sensitive macrophyte species, whilst dry weight was often the most sensitive and least variable endpoint. Even though mesocosms provide the opportunity for recovery and community responses to be observed, such information has not been used by regulatory authorities. Future regulatory mesocosm studies can build upon the shortcomings highlighted here, providing a greater chance of regulatory impact.

2.01.P Aquatic and Terrestrial Plant Ecology, Ecotoxicology and Risk Assessment

2.01.P-Tu058 Algae Species Sensitivity Distribution to Terbutylazine, Azoxystrobin, Copper and Their Mixture Effects

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Chemical risk assessments to protect the freshwater algae community often rely on a single green algal species, despite natural communities containing hundreds of taxa with diverse functional traits. This study asks how sensitivity to common pesticides vary across ecologically relevant freshwater microalgae and whether chemical mixtures interact, altering toxicity.

This study tests species from green algae, diatoms, and cyanobacteria, representing differences in morphology, surface area to volume ratios, and photosynthetic pathways. Growth inhibition assays were conducted using modified standard OECD guidelines in 96-well microplates monitoring growth as increase in light absorbance at various timepoints over 48 hrs. Species were exposed to three surface-water contaminants: the herbicide terbutylazine, the fungicide azoxystrobin, and copper chloride, both individually and in binary mixtures. Preliminary results indicate clear variation in sensitivity among taxa and chemicals, with terbutylazine being the most potent chemical across species. For *R. subcapitata*, all binary mixtures produced a slight antagonist response. Species sensitivity distributions (SSD) will be constructed from the single chemical datasets to quantify community level risk.

These findings provide mechanistic insight into functional trait based differences in pesticide susceptibility and will inform future toxicokinetic-toxicodynamic (TKTD) modeling. Overall, the work supports more ecologically realistic pesticide risk assessments and improved protection of freshwater primary producers.

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2.01.P-Tu059 Beyond Lemna and Myriophyllum – Validating Non-Standard Macrophyte Species

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Reference chemicals such as 3,5-dichlorophenol (3,5-DCP) are routinely used to verify the sensitivity of standard aquatic plant toxicity tests with *Myriophyllum spicatum* (OECD 239) and *Lemna gibba* (OECD 221). However, the responses of non-standard macrophytes to such benchmarks are still largely unknown. In this study, eight non-standard macrophyte species, including both monocotyledonous and dicotyledonous plants as well as free-floating, emergent and submergent species, were exposed to 3,5-DCP. Effect concentrations derived from standard tests with *Lemna gibba* and *Myriophyllum spicatum* were used as reference points to investigate whether the sensitivity of the various non-standard macrophyte species differs from that of the two standard species. The study supports the evaluation of additional macrophytes for potential inclusion in aquatic risk assessment and provides a reference-based framework, including a Species Sensitivity Distribution (SSD) for validating their use in future testing strategies.

2.01.P-Tu060 Chronic Effects of PFOA and Microplastics on Earthworms and Freshwater Snails: Ecotoxicological and Host-Parasite Outcomes

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The intensification of human activities has increased the presence of persistent contaminants, such as perfluorooctanoic acid (PFOA), a perfluoroalkyl substance, and polyethylene (PE) microplastics, which can affect terrestrial and aquatic organisms. This study aimed to evaluate the chronic effects of these contaminants, individually and in combination, on organisms from different trophic levels and habitats: sublethal responses in the earthworm *Eisenia andrei* and survival, reproduction, and host-parasite interactions in the freshwater snail *Biomphalaria glabrata* and its parasite *Schistosoma mansoni*.

In terrestrial assays, *E. andrei* was exposed to PFOA in control soil following ASTM E1676-2, using environmentally relevant concentrations (125, 250, 500, and 1000 µg·kg⁻¹). Each replicate contained 10 organisms for 56 days. Monitoring included weekly biomass measurements, fortnightly sampling, and analyses of cytotoxicity, oxidative biomarkers, and enzymatic activity, allowing the identification of physiological and immune responses to the contaminant.

For *B. glabrata*, exclusively chronic assays were conducted. Snails were exposed to PFOA (3 µg·L⁻¹), PE (3.4 mg·L⁻¹), or the PFOA + PE mixture under controlled temperature, light, and water quality for 21 days, after which they were split into infected (with *S. mansoni* miracidia) and non-infected groups. Both groups remained under exposure conditions for up to 100 days, allowing prolonged monitoring of survival, reproduction, and, in the infected group, cercarial release.

Results in earthworms showed alterations in coelomocyte density and profiles, suggesting immune activation and possible physiological stress. Antioxidant enzymes were modulated, indicating redox system imbalances, while acetylcholinesterase activity revealed potential neurotoxic effects depending on concentration and exposure time. In *B. glabrata*, the PFOA + PE mixture reduced cercarial release, indicating interference in the parasite life cycle. Reproductive changes and survival variations were also observed, demonstrating prolonged effects of chronic exposure.

These findings show that PFOA and PE, alone or combined, induce complex physiological responses in different organisms, with ecotoxicological implications and potential impacts on host-parasite interactions. The results highlight the need for integrated assessments to understand environmental and health risks associated with persistent contaminants from a One Health perspective.

2.01.P-Tu061 CropLife Europe Proposal for Specific Protection Goals for Non-Target Terrestrial Plants Risk Assessment

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To approve Plant Protection Products (PPPs) under Regulation (EC) No 1107/2009, the absence of unacceptable environmental effects must be demonstrated. Specific Protection Goals (SPGs) translate this General Protection Goal into practical guidance for Non-Target Organism risk assessments, including Non-Target Terrestrial Plants (NTTPs). As EFSA was mandated in 2024 to develop an NTTP Guidance Document, CropLife Europe experts have developed a proposal for SPGs for NTTPs to support this work.

The first step in setting SPGs is identifying ESS (Ecosystem Services) provided by NTTPs which are relevant for agriculture. According to EC guidance (2024), the relevant ESS assigned to NTTPs is the supporting service “habitat for species”. Protecting the ecological entity of NTTP communities ensures maintenance of this ESS. The ecological entity determines the attribute to be protected. For communities, it is proposed to protect plant species diversity and biomass. Biomass is a pragmatic attribute for the habitat function which can be addressed in testing and modelling and accordingly is preferred over species diversity. SPGs for NTTPs apply only off-field as requested by the EC mandate (2024). Accordingly, the main exposure route for NTTPs is via drift to areas beyond the field boundary. In addition, SPGs for the landscape level will be presented.

SPGs must be achievable under typical productive farming conditions. Typical agricultural practices such as soil tillage, mowing, or fertilizer-application can significantly impact NTTPs. Plant communities directly adjacent to arable fields are often species-poor and mostly contain species that are very abundant such as grasses. In this context, it has to be noted that the mandate to EFSA addresses common plant species of the agricultural landscape while rare and endangered species fall under other existing jurisdictions that are focused on

conservation. Accordingly, it is essential to take into account the baseline of what needs to be protected when setting SPGs. Otherwise, the translation of the SPGs into regulatory testing and a workable risk assessment is impossible.

SPGs for Non-Target Arthropods and soil organisms are also proposed and will be presented in separate posters at this conference.

2.01.P-Tu062 Data on 3,5-Dichlorophenol in Standard and Non-Standard Algae Species, an SSD Evaluation

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The term algae usually refers to a group of photosynthetic microorganisms, including green microalgae, cyanobacteria, and diatoms. Algae are key in toxicological studies to measure the impact of substances on aquatic ecosystems. Several guidelines (OECD, ISO, OCSPP) serve as standardized procedures to evaluate specific species, namely the freshwater species *Raphidocelis subcapitata*, *Desmodesmus subspicatus*, *Navicula pelliculosa*, *Anabaena flos-aquae*, *Synechococcus leopoliensis*, and the marine species *Skeletonema* sp. and *Phaeodactylum tricornutum*. The chemical 3,5-Dichlorophenol (3,5-DCP) is used as a standard substance to determine effective concentrations (e.g., EC50), and some data is already available for a few of the species previously listed. In this study, we aimed to compare the effects of 3,5-DCP in standard species and additional non-standard species to determine how different the effective concentrations are among the species. We also provide an analysis of the validity criteria based on the different guidelines and the role of non-standard species in further toxicological studies.

2.01.P-Tu063 Development and Relevance of Overspray Tests for Aquatic Plants

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During the latest EU renewal of glyphosate, the risk assessment for aquatic plants from foliar exposure via spray drift could not be finalised. Current ecotoxicological studies on floating plants, such as *Lemna* spp., focus on exposure via the water phase (e.g., OECD TG 221) and may therefore not consider further and concurrent direct foliar/ contact exposure conditions from spray drift onto floating or emergent aquatic plants.

Conversely, the plant risk assessment already evaluates the risk of spray drift to non-target terrestrial plants (NTTP), e.g. OECD TG 227. However, it remains unclear whether the outcome of this assessment sufficiently covers the risk to aquatic plant species through similar foliar exposure. To clarify this issue and address the pending regulatory gap, a dedicated SETAC working group was established to: (1) develop a standardized protocol for an overspray test for the standard species *Lemna* sp., and (2) determine to what extent this exposure scenario is already covered by current NTTP risk assessment.

Here we present the group's progress, including (I) gap analysis and literature review, (II) protocol development, (III) key methodological issues for harmonized conducts and (IV) consideration of an interlaboratory validation. The proposed method builds upon OECD TG 221 principles while introducing controlled overspray application consistent with OECD TG 227.

Ultimately, this effort aims to establish a scientifically robust basis for assessing direct spray exposure to floating and emergent aquatic plants, thereby enabling a two-dimensional (2D) aquatic risk assessment comparable to the NTTP-approach in addition to the already established three-dimensional (3D) aquatic assessment. The resulting protocol may support future OECD guideline developments.

2.01.P-Tu064 Ecotoxicological evaluation of lead concentrations typical of Lithuanian shooting ranges using different functional groups

Gintare Sujetoviene, Diana Miškelytė, Austra Dikšaitytė, Giedrė Kacienė and Jurate Zaltauskaite, Vytautas Magnus University, Lithuania

Shooting ranges represent persistent point sources of lead (Pb) pollution, with soil concentrations in Lithuania frequently reaching levels capable of impairing terrestrial biota. To assess the ecological risks associated with such contamination, this study evaluated the effects of Pb-amended soils corresponding to concentrations

documented at Lithuanian shooting ranges. Soils were spiked with a gradient of Pb levels reflecting both average and hotspot. Ecotoxicity was assessed using earthworms, plants, and soil microbial activity, representing different trophic levels and functional groups within the terrestrial ecosystem. The study indicated concentration-dependent biological effects across all organism groups, with significant reductions in earthworm reproduction and plant root elongation at Pb levels corresponding to heavily contaminated Lithuanian sites. Microbial activity showed marked inhibition, particularly at concentrations aligning with shooting-range hotspots, underscoring Pb bioavailability as a key driver of toxicity. The combined chemical–biological approach highlights the importance of integrating bioavailability and organism-level effects for risk characterization. This framework supports improved ecological risk assessment of shooting ranges and informs management actions aimed at mitigating Pb exposure in terrestrial ecosystems.

2.01.P-Tu065 Ecotoxicological Test Protocol for the Assessment of Reproductive Endpoints in Non-Target Terrestrial Plants under Greenhouse Conditions

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Ecotoxicological testing of plant protection products (PPP) on non-target terrestrial plants (NTTPs) under greenhouse conditions is usually performed according to OECD 208 and OECD 227 guidelines. EFSA's 2014 Scientific Opinion highlighted a potential need to assess effects on plant reproduction and a new Guidance Document for Non-target terrestrial plants is currently being developed, likely adding complexity to current risk assessment approaches.

Literature reviews and experimental studies have examined the feasibility and relevance of reproductive endpoints. Results indicate that (a) assessment in selected species is technically possible, (b) there is no consistent evidence that reproductive endpoints are more sensitive than vegetative ones, and (c) sensitivity may vary depending on factors such as PPP mode of action or plant species.

When regulatory requirements demand evaluation of reproductive effects, the lack of a standardized protocol remains a major challenge. To address this, a SETAC Plant Interest Group working team compiled available data and practical experience to develop a greenhouse test protocol for reproductive endpoints in NTTPs.

Currently, comments from external reviewers are being analyzed to refine the protocol. The final version will be made openly accessible to ensure transparency and broad usability.

This poster will present the proposed protocol and highlight key challenges and considerations, including extended test duration, species and endpoint selection, and robust statistical evaluation. These aspects are critical for reproducibility, regulatory acceptance, and scientific relevance.

2.01.P-Tu066 Effects of Detergents on Macrophyte Standard Species

Marcel Kahlke¹, **Theresa Graf¹**, Rabea Christmann¹ and Guido Gonsior², (1)GG BioTech Design GmbH, Germany, (2)Aquaculture, Ecotox, Marine Science, GG BioTech Design GmbH, Germany

Growing populations and increasing household consumptions are leading to higher use of laundry detergents, resulting in greater inputs of complex chemical mixtures into aquatic environments. Current EU regulations do not require full disclosure of all components, complicating ecological assessment. Furthermore, toxicity testing is typically only conducted on individual components rather than on the complete detergent formulation. Some substances are persistent and may negatively affect aquatic plants. Yet, regulatory assessments rely almost exclusively on *Lemna* species, while submersed macrophytes such as *Myriophyllum spicatum* remain largely overlooked. Therefore, we tested several commercial detergents, including eco-labelled products following current OECD macrophyte growth inhibition guidelines 239 and 221. Our results highlight the need for greater transparency in detergent composition and for macrophyte-specific endpoints in aquatic risk assessment. The work aims to improve understanding of macrophyte sensitivity to consumer product mixtures and to inform future discussions on ingredient transparency and aquatic risk assessment.

2.01.P-Tu067 Interactive Effects of Warming and Chemical Stressors on Freshwater Aquatic Ecosystems: Regulatory Integration and Applied Management Under Climate Change

We analyzed research from 2018 to 2025 on the impacts of pesticides and climate change on aquatic invertebrates, including mayflies, midges, and dragonflies in European surface waters. Results suggest effects on toxicity, chemical fate, community responses, and recovery, particularly within temperature ranges of 15–35°C. We furthermore compared current EU regulatory guidelines with climate data, revealing gaps in protection.

Climate warming and pesticide pollution pose combined, significant threats to aquatic ecosystems, emphasizing the importance of climate-informed risk assessments and sustainable farming practices. Elevated temperatures can increase the toxicity of insecticides through metabolic pathways, as indicated by Arrhenius coefficients, which are not yet incorporated into EU risk assessments. While the Water Framework Directive aims to establish pesticide standards, it currently overlooks temperature effects. Pesticides such as imidacloprid have been detected at concentrations exceeding regulatory limits, underlining the need for monitoring and supporting climate change mitigation. Temperature furthermore influences pesticide persistence via pathways including reduced oxygen, pH shifts, algal growth, and vapor pressure changes, affecting hydrolysis, degradation, photolysis, and volatilization, with some chemicals volatilizing more readily while others persist longer. Seasonal peaks in insecticide levels are driven by climate-dependent cycles of sorption and desorption, necessitating dynamic risk assessment approaches.

Phenological disruptions due to temperature changes can furthermore exacerbate chemical toxicity, potentially causing mismatches between insect emergence and habitat availability, which may threaten populations. Recovery of communities exposed to neonicotinoids is slow, with populations remaining depressed at concentrations above 1 µg/L for years and community structures permanently altered. Effective management across scales—such as riparian buffer zones and wetland restoration—can mitigate runoff and enhance resilience.

We therefore advocate policy updates to include mandatory chemical and biological monitoring, with future assessments incorporating climate scenarios, temperature-dependent toxicity, and heatwave impacts. Achieving the 2030 targets and EU standards requires integrated policies to reduce pesticide use, restore ecosystems, and adapt regulations to climate impacts.

Disclaimer/Disclosure:

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The views are those of the authors and don't represent their affiliations. The first author used perplexity.ai to improve the abstract, then reviewed and edited it, taking full responsibility.

2.01.P-Tu068 Multiscale Satellite–UAV Assessment of Vegetation Stress Following Chemical Exposure Events

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Chemical exposure incidents can cause rapid, spatially uneven damage to terrestrial vegetation. Conventional monitoring approaches often struggle to capture the full extent and severity of these impacts, which can lead to underestimation or misinterpretation of ecological risk. Field-based surveys are time- and labor-intensive, and when used alone they cover only limited areas. Remote sensing from a single platform can either miss fine-scale disturbance or lack the regional coverage needed to understand broader patterns. This study addresses these limitations by developing a multiscale framework that combines satellite imagery, such as Sentinel-2, with unmanned aerial vehicle (UAV) multispectral data to evaluate vegetation stress after chemical exposure. Satellite observations are used to describe broad-scale vegetation patterns before the exposure event or, if pre-event data are limited, to infer typical background conditions from nearby unaffected areas. From pre- and post-exposure images, key vegetation indices—the Normalized Difference Vegetation Index (NDVI), the Normalized Difference Red Edge index (NDRE), and the Green Chlorophyll Index (GCI)—are calculated to quantify changes in canopy condition. Index distributions are summarized using descriptive statistics, and threshold-based classes are applied to separate vegetation that appears unaffected, under stress, or clearly damaged. Comparisons across scales show that satellite data alone tend to underestimate or miss localized disturbances, such as edge effects along water bodies and small patch-level damage within fields, that are clearly visible in

high-resolution UAV imagery. Initial applications indicate that integrating satellite and UAV data improves both the spatial detail and the reliability of vegetation stress maps, with UAV data helping to validate and refine patterns derived from satellite images. The proposed framework links remotely sensed vegetation indices with spatial patterns of chemical impact and provides a practical tool for interpreting plant responses at the landscape scale, supporting plant ecotoxicology studies, ecological risk assessment, and more effective environmental management after chemical exposure events.

2.01.P-Tu069 Role of Mosses in Soil Organic Carbon Storage Under Heavy Metal Deposition in Coniferous Forests

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Traditional risk assessment methodologies often encounter difficulties due to the limited understanding of how metal pollutants impact soil ecosystems and their functions. Our study examines the long-term effects of, and recovery from, metal air pollution - specifically copper (Cu), zinc (Zn), and lead (Pb) - on soil organic carbon (C) storage within coniferous forests. The study area is located near a brass smelter in Gusum, Östergötland, Sweden, a site with a history of over 300 years of industrial activity. Notably, in 1982, the smelter was relocated 1 km away and its emissions were significantly reduced. Historically, the forests and their soils, including mosses, were thoroughly investigated from the 1970s up to 1994. This provides a unique opportunity to evaluate both the impacts of and recovery from prolonged heavy metal pollution. For this study, previously sampled sites in old-grown mixed coniferous forests were revisited. The research involved analysis of changes in moss and shrub cover, as well as the contents and isotopic signatures of organic carbon (C), nitrogen (N), and metals in soils, needles, and mosses.

Findings revealed that moss cover has shown signs of recovery and that contents of metals in mosses and the upper soil layers have decreased. However, persistent contamination remains evident in areas situated closer to 500 metres from the relocated smelter. Multivariate (Partial Least Squares regression - PLS) modelling highlighted the significant role of moss and shrub presence in total soil carbon storage and in the recovery process from air pollution.

This study is focused on improving methods for ecological risk assessment in metal-contaminated soils by employing site-specific strategies. This comprehensive onsite evaluation bridges a critical knowledge gap, as it challenges the common practice of removing contaminated soils without accounting for the ecosystem's inherent ability to recover from long-term metal pollution. Furthermore, the study underscores the vital role played by ground-covering mosses as a driver in ecosystem restoration and soil carbon dynamics within coniferous forests - an often-overlooked group of plants that are notably sensitive to metal contamination.

Keywords: Soil organic matter, Heavy metal pollution, Stable isotopic signature, Ecosystem modeling

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2.01.P-Tu070 Sensitivity of Aquatic Macrophytes to Copper: Comparing Functional and Structural Endpoints

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This study assessed copper toxicity in six submerged macrophyte species representing diverse ecological traits and carbon acquisition strategies by comparing two endpoints: functional (oxygen production as a proxy for photosynthesis) and structural (relative growth rate). This work aimed to determine how endpoint selection (functional vs structural) and exposure duration shape the estimation of copper toxicity in macrophytes. Static 14-day tests were conducted with five copper concentrations (11–900 µg/L), and responses were

normalized to controls. Dose–response curves were modeled using a two-parameter log-logistic function to estimate EC_x values (EC₅₀). Species sensitivity distributions (SSDs) were then derived for both endpoints to calculate protective concentrations (HC₅). Functional indicators consistently exhibited earlier and stronger responses than structural ones. At day 7, oxygen production in the species *Elodea nuttallii* showed the highest sensitivity (EC₅₀ = 19 µg/L), while growth remained largely unaffected. By day 14, inhibition intensified across most species, confirming cumulative stress under static exposure. SSD analysis reflected these patterns: HC₅ values for oxygen were 22.7 µg/L (day 7) and 27.9 µg/L (day 14), compared to 38.2 and 72.2 µg/L for relative growth rate. These findings underscore the relevance of functional endpoints for early detection and their integration into ecological risk assessment frameworks.

2.01.P-Tu071 Terrestrial Eco-toxicological Effects of Typical Tire Rubber Antioxidants and Derivation of Their Environmental Criteria

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The tire rubber antioxidant N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and its transformation product, 6PPD-quinone (6PPD-Q), are emerging contaminants of concern. Although their toxicity toward aquatic organisms has been widely documented, their ecotoxicological effects on terrestrial species remain poorly understood, hindering the development of terrestrial environmental criteria due to a lack of relevant toxicological data. To address this gap, this study evaluated the toxicological effects and uptake-translocation mechanisms of 6PPD and 6PPD-Q in rice plants (*Oryza sativa*). In parallel, we compiled existing aquatic toxicity data for both compounds to derive their short- and long-term water quality criteria (WQC). Using both root and foliar exposure assays, we observed that 1 mg/L 6PPD and 0.5 mg/L 6PPD-Q induced significant phytotoxicity in rice, affecting key metabolic pathways and impairing growth traits. Subsequent analysis revealed that 6PPD exhibited a higher bioaccumulation potential in both roots and leaves. In contrast, 6PPD-Q showed limited translocation from shoots to roots under foliar exposure, with a pronounced tendency to accumulate in leaf tissues.

To supplement gaps in acute toxicity data for 6PPD-Q, we applied quantitative structure-activity relationship (QSAR) and interspecies correlation estimation (ICE) models. Using a species sensitivity distribution (SSD) approach, short-term and long-term WQC values were derived. The WQC for 6PPD were 26.02 µg/L (short-term) and 4.30 µg/L (long-term), suggesting an acceptable level of ecological risk. In contrast, the stringent WQC for 6PPD-Q — 0.20 µg/L (short-term) and 0.15 µg/L (long-term) — underscore its substantial ecological risk, highlighting an urgent need for regulatory attention.

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2.01.P-Tu072 Testing Aquatic Macrophyte Growth Inhibition in 10 Species Simultaneously - Combining SSD and Time-variable Exposure Outdoors.

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Aquatic plants are sensitive to herbicides. Standard laboratory tests expose single species to constant concentrations of herbicide. Whereas our outdoor study focused on effects in multiple species for realistic time-variable concentrations. The study was based on OECD Guideline 239: Water-Sediment-Macrophyte Toxicity Test. Growth inhibition compared to controls was determined in 10 species for 14 days. The test established an EC₅₀ for each species and a Species Sensitivity Distribution (SSD) under realistic exposure. We present results showing that OECD validity criteria were satisfied, providing robust growth endpoints. Time-variable profiles were characterised by chemical analysis to enable comparison with FOCUS-modelled exposures in edge-of-field waters.

2.01.P-Tu073 The Effect Testing Toolbox for Non-Target Terrestrial Plant Risk Assessment: what is in and what is missing?

Sebastian Fellmann¹ and Rena Jutta Irene Isemer², (1)*Bayer AG, Germany*, (2)*Crop Science, Bayer CropScience AG, Germany*

The standard European environmental risk assessment (RA) for plant protection products in the area of Non-Target Terrestrial Plants (NTTPs) follows the Guidance Document on Terrestrial Ecotoxicology and is based on two OECD test methods (OECD 208&227). In 2014, the European Safety Authority (EFSA) published the Scientific Opinion addressing the state of the science on risk assessment of plant protection products for NTTPs where additional and/or higher tier testing options were discussed for their relevance for the NTTP RA. The list comprises pre-screening, open literature and efficacy data, and refined laboratory, semi-field and field studies. Testing reproductive parameters, non-crop (wild) species, and plant or species interactions were also discussed. No harmonized test guidelines are available for these additional studies. Since 2014, novel NTTP study designs have been developed that may support refining the RA and/or informing an NTTP reference tier. An NTTP Effect Testing Toolbox comprising laboratory bioassays, greenhouse studies, semi-field and field trials, and predictive modeling approaches was assembled. The present work provides a first attempt at systematically analysing the NTTP Effect Testing Toolbox regarding advantages and disadvantages of study designs based on whether they are fit-for-purpose, feasible, and proportionate. Some novel methods should be taken into account for the mandated Terrestrial Guidance Document review by EFSA. As the availability of harmonized test methods is key, this was evaluated as well and showed a gap for standardized methodologies for higher tier assessments and improved predictive modeling techniques, along with collaborative efforts for data sharing across Europe. We propose integrating intermediate tier testing and modeling methods to balance precision with ecological relevance. The size of the assessment factor used to address remaining uncertainties in the NTTP RA is expected to be discussed in the Terrestrial Guidance Document review by EFSA. To define an assessment factor, a reference tier for a sufficient number of plant species and plant protection products is needed. The NTTP Effect Testing Toolbox is evaluated regarding usability of the listed study designs to deliver a reference tier. Further work, involving all stakeholders, is needed.

2.01.P-Tu074 The Impact of Selected Chemicals on Various Species of Freshwater Alga and Cyanobacteria

Katarzyna Winiarska and Joanna Gilowska, Lukaszewicz Research Network - Institute of Industrial Organic Chemistry, Poland

The increasing number of chemical substances released into the environment poses a significant challenge for aquatic ecosystems. Many of these compounds can affect primary producers, disrupting ecological balance and food web dynamics. Algae, as key organisms in aquatic ecosystems, are highly sensitive to chemical stressors and serve as reliable indicators of environmental toxicity. They form the basis of the aquatic food chain, directly supporting herbivorous organisms.

In this study, we assessed the inhibitory effects of selected chemicals on the growth of freshwater green algae and cyanobacteria under controlled laboratory conditions. For each group, four chemical substances were tested using standardized growth inhibition assays. The No Observed Effect Concentration (NOEC) values were determined for all tested compounds, and concentration–effect relationships for yield and growth rate were analyzed to illustrate species-specific responses.

The tests were conducted in accordance with OECD Guideline 201 for algal growth inhibition, but using species other than those recommended in the guideline: *Synechococcus leopoliensis* (cyanobacteria), *Chlorella vulgaris* and *Tetrademus obliquus* (green algae). Our results revealed pronounced differences in tolerance between taxonomic groups, with cyanobacteria being less sensitive than green algae. These findings contribute to a better understanding of chemical stressors in aquatic ecosystems and support the development of more accurate environmental risk assessment strategies.

2.01.P-Tu075 The Intriguing Use of Plant Visual Injury Symptoms in Ecotoxicology

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Current regulatory testing for non-target plants is based on the test guidelines from OECD and the OCSPP 850.4000 series.

Qualitative observations of plant visual injury (PVI) by the unaided eye have been included in non-target terrestrial plant (NTTP) and macrophyte studies for many years. PVI document observations of effects after treatment with plant protection products in addition to the standard quantitative parameters (weight, height etc.). Historically, PVI was developed for screening of plant health based on methodology evolved from Weed Scientists with deep expertise in PVI over an extensive range of terrestrial plant species. As PVI is quicker to assess than biomass / height, the Tier 1 OECD 208/227 (and respective OCSPP guidance) also use PVI for PPPs to determine if a Tier 2 study is required (>50% effect trigger). PVI provide evidence to support that

quantitative injury is indeed caused by the plant protection product and not unrelated stresses such as disease, insects or nutrient deficiency.

Recently, member states from the European Central Zone made requests for statistical calculation of ECx for NTTP PVI endpoints to be used in the risk assessment. This reflects a precautionary approach addressing the EU concern that existing quantitative endpoints potentially may not fully cover the risk to plants. However, statistical calculation of ECx PVI within the studies may create a mismatch with the OCPPTS 850.4000 series which generally considers visual ratings as qualitative data.

We understand that there is a great uncertainty about what the PVI results reported in NTP studies mean for plant health and how these results can best be considered in the risk assessment. However, while PVI ratings appear quantitative when expressed in percent damage, they are in fact closer to an ordinal scale and calculating ECx values from visual ratings may violate assumptions for dose-response models (which is the topic of a separate poster).

The main value of PVI ratings is that it can provide quick and valuable information for the interpretation of the study results. However, guidance and standardized, ring-tested procedures are missing for NTTP and aquatic macrophytes.

Therefore, the poster will focus the discussion on how PVI could be classified and contextualized alongside the quantitative endpoints of the studies for an improved assessment and use in risk assessment.

2.01.P-Tu076 The new OECD Test Guideline No. 239 - a Comparison with the Previous Version

Maren Dill¹ and **Guido Gonsior²**, (1)GG BioTech Design GmbH, Germany, (2)Aquaculture, Ecotox, Marine Science, GG BioTech Design GmbH, Germany

The OECD test guideline 239 describes the water-sediment toxicity test with the rooted aquatic plant *Myriophyllum spicatum*. On June 25th in 2025 a revised version was published. Further, there are still ongoing discussions about additional parameters.

We present an historical insight based on the conducted ring tests and discuss the most relevant differences of the two versions.

2.01.P-Tu077 TKTD modeling of semi-static algae tests with time-variable herbicide exposure – data handling and evaluation of statistical endpoints

Natalie Albrecht¹, **Tido Strauss¹**, **Kim Rakel¹**, **Eric Bruns²**, **Jutta Hager²**, **Thomas G. Preuss²**, **Seamus Taylor³** and **Johannes Witt²**, (1)gaiac eco assessment GmbH, Germany, (2)Bayer AG, Germany, (3)ADAMA, United Kingdom

Ecotoxicological tests with planktonic algae are an integral part of an informed risk assessment of plant protection products (PPPs). Recent test developments now also allow the evaluation of toxic effects of time-variable peak exposures. Toxicokinetic-toxicodynamic (TKTD) models are increasingly being used to investigate and predict the effects of time-varying exposure concentrations of PPPs on aquatic organisms and can now also be applied to these refined algal tests.

We conducted peak exposure laboratory studies with the green alga *Raphidocelis subcapitata* exposed to the herbicide diflufenican, using a semi-static approach with a series of filtration and resuspension steps to examine exposure patterns with varying peak duration and intervals based on the OECD guideline 201 (Freshwater Alga Growth Inhibition Test). We applied the software tool ‘cvasi’ (calibration, validation and simulation of TKTD models in R) to evaluate the experimental algae data. Due to their exponential growth in unlimited tests, phytoplankton often show biomass increases over two orders of magnitude, raising the question of logarithmic data transformation.

We will show experimental results of a test series and the TKTD model calibration and validation outcome and discuss the effect of data transformation on different statistical model quality endpoints.

2.01.P-Tu078 Visual Injuries in Aquatic Plants: Gaps, Challenges, and Recommendations from a retrospective Assessment of Study Records

Katrin Kuhl¹, **Bastian H. Polst²**, **Gertie Arts²** and **Andreas Solga¹**, (1)Bayer AG, CropScience, Germany, (2)Wageningen University and Research (WUR), Netherlands

Visual injuries (phytotoxicity) in aquatic plants are gaining increasingly regulatory interest in the risk assessment of plant protection products. Yet a clear, validated evaluation scheme is still lacking. To support the development of new protocols, experimental approaches, and evaluation schemes, we analyzed existing registration study records available at Bayer AG, covering more than 500 GLP *Lemna*-studies dating back to the

1980s.

Our aim is to evaluate which types of phytotoxic effects have been observed, which assessment scales (qualitative vs. quantitative) were applied, and how severity and prevalence were recorded across studies. This allows us to identify evaluation schemes that have proven effective and robust, as well as reveal gaps in past and current assessment practices.

We found a general lack of quantitative data, likely caused by the omission of quantitative assessments in the respective test guidelines (e.g. OECD 221). Visual injuries were mostly recorded qualitatively and often without a clear indication of severity (e.g., slight, moderate, strong) or prevalence (e.g., number or share of affected fronds). In several cases, ordinal scales (e.g. for frond color: dark green, light green, yellow) were used without adequate context or differentiation to related injuries (e.g., chlorosis). Understanding these subtle differences is crucial for enabling a quantitative comparison with conventional (e.g., frond number, dry weight) endpoints. Based on these insights, we provide first recommendations towards a harmonized assessment framework for visual injuries in aquatic plants and highlight the need to relate visual injuries to ecologically relevant effects impacting growth and survival of aquatic plants. Upon completion, the assessment of our dataset will help to define standardized criteria for assessing visual injuries, enable quantitative evaluation of severity and prevalence, and improve consistency in future regulatory assessments.

2.01.P-Tu080 Investigating Alternative Treatment Strategies for Harmful Algal Blooms

Andrew Barrick¹, Ben Beck² and Tham C. Hoang¹, (1)Auburn University, (2)USDA-ARS, Aquatic Animal Health Research Unit, United States

Copper-based herbicides are commonly used to treat harmful algal blooms (HABs) as they are more effective and affordable than other treatment options. A limitation of copper is that it rapidly complexes with organic material in aquaculture ponds and precipitates into sediment. Over time, copper can accumulate and contaminate pond systems. This presentation outlines research conducted at Auburn University that explores alternative treatment approaches for treating cyanobacteria. This includes characterizing the efficacy of organic herbicides and developing high throughput testing to explore whether herbicides mixtures were more effective than copper sulfate. Two cyanobacterial genera, *Anabaena* and *Microcystis*, which commonly bloom were treated with 15 different herbicides to identify efficacy. Herbicide mixtures were tested using high-throughput screening to determine if mixtures were more effective than single herbicides alone. Results identified five alternative chemicals (bispiribac sodium, diuron, diquat, hydrogen peroxide, and penoxsulam) that reduced cyanobacterial growth with diuron and penoxsulam being effective at a low concentration for *Anabaena* and diuron being effective against *Microcystis*. Mixture studies demonstrated that most copper: organic herbicide mixtures were either antagonistic or additive and copper: diquat mixtures were synergistic. Results of the study suggested that diuron and penoxsulam may be effective alternatives to copper, however considerations need to be made regarding their environmental impact. The study also identified that while copper: diquat mixtures were more effective, increases in efficacy were likely not offset by the cost of the treatment approach when compared to copper.

2.01.P-Tu081 Toxic Effects of Ciprofloxacin and Sulfamethoxazole on Crustaceans, a Marine Diatom, and a Freshwater Plant

Marianna Pauletto, Edoardo Pietropoli, Rebecca Zgheib, Marco Selmo, Giacomo Melotto, Rosa Maria Lopparelli, Lorena Lucatello and Marco De Liguoro, Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Italy

Pharmaceuticals are essential for human and animal health, but when excreted or improperly disposed of, they can harm the environment. Antibiotics like ciprofloxacin (CIP) and sulfamethoxazole (SMX) are particularly harmful as they persist in surface waters, where they can harm non-target organisms, including crustaceans, algae and aquatic plants. To provide a clearer understanding of the environmental impacts of these antibiotics, the present study investigates the toxicity of CIP and SMX on two primary consumers, the crustaceans *Daphnia magna* and *Artemia salina*, and two primary producers, the freshwater plant *Spirodela polyrrhiza* and the marine diatom *Phaeodactylum tricornutum*.

Experimental procedures followed OECD and ISO guidelines, when available, with species-specific adjustments. Toxicity tests included algal and duckweed growth inhibition, acute crustacean immobilisation with 10-day post-exposure follow-up, and embryo hatching assays. Endpoints measured encompassed growth, survival, morphological abnormalities, and frond/root metrics via microscopy and image analysis. CIP and SMX stability were confirmed by analysing drug-solution samples collected at the beginning and end of each assay. SMX inhibited *P. tricornutum* growth with an EC₅₀ of 2.69 mg/L²¹, while CIP had no effect. On *S. polyrrhiza*, EC₅₀s ranged 0.77–2.52 mg/L²¹ for SMX and 0.04–0.34 mg/L²¹ for CIP. *A. salina* was largely resistant, though embryos exposed to both antibiotics showed some non-lethal malformations. *D. magna* embryos were moderately sensitive, with EC₅₀s of 41.27 mg/L²¹ (SMX) and 95.93 mg/L²¹ (CIP) and malformations similar to those observed in the brine shrimp. CIP exhibited marked delayed toxicity in *D. magna* juveniles, with EC₅₀

dropping from 47.12 mg/L¹ at 48 h to 14.98 mg/L¹ after 10 days. Finally, both antibiotics slightly reduced daily growth.

Both antibiotics can impact aquatic organisms. *S. polyrhiza* appeared to be the most sensitive organism, while *P. tricornutum* was affected only by SMX. *A. salina* remained largely unaffected, whereas *D. magna* showed intermediate sensitivity. Environmental concentrations, especially of CIP, exceed predicted no-effect levels, highlighting ecological risks to freshwater ecosystems. These results underscore the need for sensitive plant endpoints, extended toxicity tests, and consideration of chronic and mixture exposures in future assessments.

2.02 Combining and Integrating Sustainability and Risk Assessment from Environmental, Social, and Economic Perspectives

2.02.T-01 Towards Robust Decision-Making in Safe and Sustainable by Design (SSbD): Current State and Recommendations for MCDA Integration

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The Chemicals Strategy for Sustainability (CSS) outlines policies towards a toxic-free environment. It promotes Safe and Sustainable by Design (SSbD) as a pre-market approach to chemicals to deliver their essential functionality, but avoid potentially harmful volumes and properties at risk to human and environmental health.

Developing alternative chemicals, materials, or products under the SSbD paradigm requires comprehensive safety and sustainability assessment and the integration of potentially diverging stakeholder perspectives along the life cycle. Integrating decision-support methods like multi-criteria decision analysis (MCDA) is thus advocated, to support comprehensive evaluation over a broad range of criteria and potential trade-offs.

However, guidance is needed for decision-making in complex assessments encompassing a broad scope of chemicals, materials, or product life cycles, a potentially wide range of alternative designs, and numerous safety and sustainability criteria. This study adds to the foundation for future recommendations to guide the implementation of MCDA in SSbD assessments. By reviewing a set of 18 papers performing safety and sustainability assessments together with MCDA, we address the following question: How are various decision-making features (i.e., characteristics that define the MCDA process) present in current studies on safety and sustainability assessment in combination with MCDA, and which aspects require attention for the development and application of MCDA in the SSbD framework?

Our findings reveal six features that are notably essential to address, as, e.g., clearly defining the problem statement and the decision-maker, and justifying the set of criteria and alternatives, for selecting an appropriate MCDA method to the specific problem. At present, these features are insufficiently addressed in literature, and even methodologically unsound combinations of MCDA methods are used. These are main reasons for the call for dedicated guidance on SSbD-related problem characteristics, that are of importance for selecting an MCDA method to ensure suitable decision-making recommendations. Addressing the presented features could help charting the pathway towards methodologically aligned studies, sound results, and appropriate decision support.

In this presentation, we share our insights and outline key decision-making features. Additionally, we provide recommendations for adopting a more structured approach to MCDA in SSbD.

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2.02.T-02 Treating Safe and Sustainable-by-Design as a Multi-objective Optimization Problem – Mapping Tradeoffs Using Decision Spaces of Polyethylene Terephthalate and Polylactic Acid

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The European Commission's Safe and Sustainable-by-Design (SSbD) framework promotes the integration of human and environmental safety with holistic sustainability assessments. While research combining risk assessment (RA) and life cycle assessment (LCA) is well established, few studies have applied a multi-objective optimization problem (MOOP) approach. Thus, this work formally introduces the first SSbD-MOOP method and demonstrates its application through a case study.

In the case study, a hypothetical product designer compares fossil-based polyethylene terephthalate (PET) and bio-based polylactic acid (PLA) as material options. Over 35,000 lifecycle scenarios were simulated, evaluating sustainability via global warming potential and safety via plastic pollution impacts according to the PPI100 method. The analysis revealed lifecycles with trade-offs between safety and sustainability, as well as scenarios that optimize both through end-of-life recycling. To ensure meaningful material-level comparisons, the study introduces the concept of SSbD decision spaces, highlighting the role of PLA's potential biodegradability when assessing its relative performance to PET. For product designers, this analysis yields several practical recommendations: material choice and end-of-life strategies are critical; circularity should be prioritized; biodegradable polymers should also be recycled; landfill-only markets should be avoided for PLA; and materials should be matched to specific use requirements.

Compared to traditional LCA-RA integration methods, the SSbD-MOOP approach provides a distinct advantage by visually mapping all possible safety and sustainability trade-offs, supporting more informed decision-making. This represents the first structured attempt to embed MOOP within the SSbD framework, with future refinements encouraged to broaden its applicability across diverse innovation contexts.

2.02.T-03 Assessment of Functional Alternatives to Fluorinated Foam Blowing Agents in Insulation Materials

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By taking the example of the substitution of fluorinated gases used as foam blowing agents in insulation materials as a case study, here we aim to apply and adapt a well-known multi-criteria decision analysis (MCDA) method used for chemical alternatives assessment known as the multi attribute utility theory (MAUT) approach. 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 MAUT approach was used to compare the different alternatives. Four decision-making scenarios were defined in order to illustrate the flexibility of the MAUT method for the assessment of functional alternatives. Overall, 32 alternative materials to polyethene foams (also known as polyethylene foams) and extruded polystyrene foams containing fluorinated gases were identified. 9 insulation materials were shortlisted for further evaluation based on the amount of data available. Overall, alternatives ranked better than polyethene foams and extruded polystyrene foams in every decision-making scenario tested in this study, suggesting that suitable and safer alternatives to fluorinated gases used in insulation foams can be identified. This work highlights how the choices made by the decision-maker to develop a MAUT model influence the final ranking of the alternatives being evaluated. This might be highly relevant in a regulatory context as the availability of suitable alternatives is a critical part in the decision-making on bans of harmful substances. Although promising in the field of alternatives assessment in a regulatory context, further work is needed to develop appropriate guidance for using MAUT methods to identify suitable alternatives to substances of concern.

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2.02.T-04 Are Larger Companies More Sustainable? Quantifying Scaling Relationships Between Company Size And Environmental Indicators

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Quantifying the environmental impact of companies, such as CO₂ emissions, is crucial for assessing their sustainability. Understanding the relationship between company size and environmental indicators can help estimate sustainability impacts. While the importance of "scaling up sustainability" is widely recognized, the fundamental question of how to estimate the sustainability for companies of varying sizes remains. We used data from 14,342 companies worldwide and characterized the scaling relationships for four company size indicators (e.g., revenue, assets, and employees) and 13 environmental indicators (e.g., raw material, CO₂ emission, and total waste). The slopes and coefficients were estimated based on the mixed-effect model. For most environmental indicators (nine out of 13), scaling exponents did not significantly differ from one, indicating that small and large companies perform equally per unit of revenue (€). In addition, accounting for the economic sector of companies improved the explanatory power of the model. This shows that sector-specific characteristics are key in estimating environmental indicators based on company size. This system thinking perspective can hopefully help organizations systematically navigate development interventions involved in tackling scales.

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2.02.P Combining and Integrating Sustainability and Risk Assessment from Environmental, Social, and Economic Perspectives

2.02.P-We089 Early-Stage Sustainability Assessment for Bio-Based Benzoxazines Using LCA and Safety Screening

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Bio-based materials offer a promising and sustainable alternative to materials derived from non-renewable resources, reducing the dependence on fossil fuels. However, it is essential to assess their sustainability during the early design stages to ensure they deliver lower environmental impacts than conventional fossil-based products.

This study demonstrates the integration of life cycle assessment (LCA) into the ecodesign of novel bio-based benzoxazine resins, supporting their development under a Safe and Sustainable by Design (SSbD) approach. First, a hotspot analysis of predominantly fossil-based benzoxazine formulations was conducted to identify key environmental impact drivers and prioritise redesign efforts. Next, a screening of promising bio-based feedstock chemicals was performed to evaluate their environmental profiles and select sustainable candidates for new formulations. Finally, environmental impact data were combined with chemical safety considerations to establish the first iteration of the SSbD framework for benzoxazine resins.

The results reveal potential environmental benefits of bio-based chemicals compared to fossil-based counterparts when substituting fossil-based chemicals with bio-based alternatives, while also highlighting possible trade-offs and critical knowledge gaps that must be addressed to achieve truly safe and sustainable formulations. This work contributes to advancing bio-based resin development by embedding environmental and safety considerations into early-stage design. The research was carried out within the Horizon SSSUCHY-Next project (grant agreement No. 101157517), which aims to develop sustainable and circular high-performance bio-based composites.

Disclaimer/Disclosure:

The research was carried out within the Horizon SSSUCHY-Next project (grant agreement No. 101157517).

2.02.P-We090 Advancing Integrated Sustainability Assessment of chemicals: combining LCA, Risk Analysis and Tools for Safer Industrial Processes

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Industrial sectors face increasing pressure to align with climate neutrality and pollution prevention targets for 2030 and 2050. Achieving these goals requires methodologies that integrate environmental sustainability assessment with chemical risk management, ensuring compliance with regulatory frameworks such as REACH (Reg. EC 1907/2006) and CLP (Reg. EC n. 1272/2008) and the EU Chemicals Strategy for Sustainability (CSS). This work presents a technical approach for embedding sustainability principles into industrial decision-making by combining Life Cycle Assessment (LCA), hazard-based risk assessment, and sustainable platforms for data integration. The proposed framework enables comparative evaluation of substances and technologic processes across multiple aspects based on the One Health approach and related implications.

Methodologically, the framework identifies prospective LCA to foresee impacts of emerging technologies, integrates Best Available Techniques (BAT) benchmarks, and applies predictive analytics also to support substitution strategies. Greentech plays a key role in harmonizing heterogeneous datasets, improving transparency, and reducing uncertainty.

The case of chemical sector demonstrates how this integrated assessment can identify trade-offs between environmental performance and regulatory compliance, guiding also the adoption of Safe and Sustainable by Design (SSbD) principles. Results highlight critical implementation challenges, including data interoperability, methodological harmonization, and alignment with policy objectives.

In addition to presenting the methodological framework, this contribution introduces the Special Issue “Towards Sustainable Industrial Processes by 2030 and 2050: Technologies, Regulations and Implementation Challenges”, which aims to address research and case studies on regulatory drivers, technological innovation, and environmental implications of the sustainable transition.

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2.02.P-We091 Integrating LCA-RA Methods for Environmental Sustainability Assessment of Pharmaceuticals: Supporting Safe and Sustainable by Design and Decision-Making

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The ambition to green the pharmaceutical sector requires a system to assess the environmental sustainability of pharmaceutical products. Such a system could build upon existing assessment methods such as Life Cycle Assessment (LCA) and Risk Assessment (RA). LCA focuses on comparative metrics to quantify upstream and midstream environmental burdens, including climate change, resource use, and human toxicity, while RA uses absolute metrics to evaluate exposure, persistence, bioaccumulation, and (eco)toxicity of pharmaceuticals. Stakeholders from regulatory agencies, industry, and healthcare emphasize the need for a transparent and scientifically robust framework that supports decision-making in product development, regulatory review, procurement, and health system planning.

The EU TransPharm project has developed a harmonised Environmental Sustainability Assessment Framework that combines elements of LCA and RA methods, recognizing that both life-cycle impacts and (eco)toxicological risks are critical for comprehensive evaluation. Its modular design allows adaptation of system boundaries, impacts, and aggregation levels to diverse decision contexts, while also providing general guidance for broader applications. The framework should support future implementation of Safe and Sustainable by Design approaches and be compatible with Multi-Criteria Decision Analysis.

The framework is built on three methodological pillars: Goal Definition, System Boundary Mapping, and Impact Quantification. Goal Definition distinguishes assessments at product level, therapeutic strategy level, or process optimization level, guiding selection of functional units, data requirements, and modelling assumptions. System Boundary Mapping aligns cradle-to-gate, gate-to-gate, cradle-to-grave, or use-to-EoL scopes with LCA

and RA requirements. Impact Quantification integrates LCA impact categories with RA metrics to evaluate trade-offs across sustainability, safe objectives. Applied to pre-screened research questions, comparison of products, evaluation of treatment options, and optimization of processes, the framework integrated LCA-RA methods advances environmental sustainability assessment in pharmaceuticals while fostering innovation, regulatory alignment, and responsible patient care. To demonstrate its applicability, the presentation will show assessment results comparing batch and continuous production, also region-specific environmental risk comparisons for selected pharmaceuticals.

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2.02.P-We092 Defining Minimum Data Quality for Decision-Making with Integrated Sustainability Assessment of Pharmaceutical Products: A Case Study on Anti-Inflammatory Drugs

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To prevent damage now and in the future, it is essential to make choices that reduce environmental impacts. This requires combining different assessment methods. The integration of life cycle assessment (LCA) and risk assessment is increasingly important for evaluating the environmental sustainability of pharmaceuticals, particularly as these evaluations may influence procurement, reimbursement, and (clinical) decision-making. However, the reliability and comparability of such integrated assessments depend critically on the quality and completeness of underlying Life Cycle Inventory (LCI) data and up-to-date impact assessment calculations. In the pharmaceutical sector, detailed primary data are often lacking in all life cycle stages, even for well-studied active pharmaceutical ingredients (APIs) like non-steroidal anti-inflammatory drugs (NSAIDs). As a result, sustainability assessments frequently rely on data from patents, literature, or other approximations.

This poster presentation examines to what extent such estimated LCI data are sufficient for robust (clinical) decision-making, using NSAIDs as a case study. We will apply new LCA methods developed in the EU Project TransPharm in the decision-making context by systematically testing impacts of patent-derived and other estimated LCI data, and by applying guidance for integrated assessment of pharmaceuticals. This work shows how minimum LCI data quality requirements can be defined and validated for pharmaceuticals, specifying which uncertainties should be addressed and reduced in order to fit the purpose of the integrated assessment. The implications for decision-making based on multiple sustainability criteria and further harmonization of assessment approaches will also be discussed. By providing concrete case study results for multiple APIs, we aim to support the development of integrated assessment tools that are both scientifically robust and practically applicable for healthcare and policy decision-makers.

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2.02.P-We093 An Integrated Environmental Risk Assessment Framework for Safe and Sustainable Design of Pharmaceuticals: When Lower Persistence Does Not Trade Off Efficacy

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Pharmaceutical residues in freshwater environments are a global concern. In response, the European Union funded the project TransPharm (<https://transforming-pharma.eu/>) following the 'Benign by Design' concept for developing novel fluoroquinolone (FQ) antibiotics in order to mitigate the environmental persistence of traditional FQs. This study introduces an interdisciplinary framework for comparative environmental risk assessment (ERA), applied to a new FQ derivative, ciprofloxacin-hemi (CIP-hemi), in comparison to its conventional counterpart, ciprofloxacin (CIP). This framework integrates multiple dimensions, i.e., antibiotic efficacy, human pharmacokinetics, environmental degradability, and ecotoxicity to non-target organisms. By combining experimental data from microbial assays, in vitro safety tests, and ecological toxicity studies, with in silico physiologically based pharmacokinetic (PBPK) modelling, the framework aims to assess trade-offs between human health safety needs (i.e., therapeutic efficacy) and environmental protection (i.e., risk to freshwater ecosystems).

Using this framework, we show that – when the two antibiotics reach equivalent efficacy levels – the expected environmental risk to aquatic ecosystems (RQeco) caused by CIP-hemi exceeds that of CIP in most investigated scenarios. Although CIP-hemi degrades faster than CIP through hydrolysis in human urine, and shows lower ecotoxicity to the sensitive non-target organism *Microcystis aeruginosa*, the minimum inhibitory concentrations (MICs) of CIP-hemi are 17-71 times higher than those of CIP for clinically relevant pathogens. As a result, higher doses of CIP-hemi are required to achieve equivalent therapeutic outcomes, making the RQeco of CIP-hemi surpassing that of CIP. Only under highly specific conditions, i.e., intravenous administration at clinically unrealistic high doses, CIP-hemi shows the potential for lower risk than CIP regarding the spread of antimicrobial resistance (RQMIC).

Our study indicates that prioritizing degradability alone is not sufficient in designing environmentally safe and sustainable pharmaceuticals. Instead, the interdisciplinary approach developed in this study is essential to offer a more robust basis for identifying antibiotic derivatives that are both clinically effective and environmentally responsible.

2.02.P-We094 Life cycle risks and environmental Impacts of consumer products and their chemicals on human health and ecosystems: The integration of USEtox 3 within GLAM applied to BPA and alternatives in toys

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The ubiquitous presence of more than 100,000 chemicals in thousands of consumer products stresses the need for screening a broader set of chemicals. The USEtox model was first extended to enable user to calculate characterization factors for human and ecotoxicity exposure to chemicals during product use. Once USEtox is integrated within the Global Life Cycle Impact Assessment Method (GLAM) developed by the UNEP Life Cycle Initiative, it enables users to compare human toxicity and ecotoxicity impacts to other impacts of the considered product on human health (HH), ecosystem quality (EQ) and socio-economic assets (SEA), and to weight these using econometric and MCDA-based weighting factors. The approach is illustrated by studying the impacts BPA & alternatives in toys, comparing toxicity to other impacts of the toys production.

To cover a wide range of chemicals-products combinations, we developed complementary models for: a) the object interior model – for chemicals in toys & building materials, b) the food contact model, c) the object surface model, d) The skin surface layer for personal care products. Substance coverage was substantially extended for non cancer effects from less than 750 regulatory chemicals to experimentally derived data for more than 5000 chemicals.

We assessed the exposure to BPA and alternatives across representative toy archetypes, identifying dominant exposure pathways, and most impacting type of toys. The highest total BPA exposure mass for a child occurs between ages 6 and 11. Summing across age groups, the aggregate exposure mass of BPA for a child from 6 months to 11 years is estimated to be 13.4 mg. We also evaluated health risks by comparing exposure doses to toxicity benchmarks. None of the aggregate exposures exceeds the corresponding RfD. However, dermal exposure to BPA is only a factor of 4 lower than the RfD for rep/dev effects.

Taking the doll and teething ring as examples, we assess the life cycle impacts associated with toy production. Toxicity damages on human health expressed in DALYs can be substantial compared to all other human health relevant categories. Once USEtox is integrated into GLAM, it enables us to compare human toxicity and

ecotoxicity impacts to another 15 impact categories. This approach consistently operationalizes the inclusion of chemicals in products during use for LCA and shows that these exposures are substantial and can be higher than the impacts of toxics over the rest of the life cycle.

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2.02.P-We095 Environmental Indicator of Pesticide Uses

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How to make agriculture and horticulture more sustainable? This question is part of an extensive societal debate. A safe and sustainable production of food and ornamentals is asked for. This requires that farmers and growers use well informed crop protection strategies. The environmental indicator crop protection (EICP) is being developed to inform farmers and growers about the environmental impacts of their pesticide use and to enable the comparison of strategies. By making use of the extensively developed field of prospective risk assessment in the pesticide regulation, the methodology is drawn upon and consistent with the European Food Safety Authority guidelines. The EICP has been developed based on existing environmental risk assessment guidance and models, using the lower tier risk assessment of pesticides as the basis. The EICP is multidimensional and calculates impacts on non-target organisms including pollinators, in-soil organisms, aquatic organisms, birds and mammals and non-target arthropods. All risk indicators are expressed by an exposure toxicity ratio (ETR) per unit area of agricultural land. Only active ingredients and products are assessed that have been approved. Properties of the active ingredients of these products have been stored in a pesticide properties database. They can be selected by the user and the tool will thereafter quantify the impact for the relevant protection goals. The tool also includes options for impact reduction such as the use of drift reducing techniques.

The methodology of the EICP that was originally developed for the Netherlands will be extended for use to other countries (EU and worldwide). Also additional exposure routes will be added such as runoff and drainage. The further development of the tool is a joined effort of private sector and public parties and research organisations with the aim to make the tool available for farmers and other private sector parties. The EICP could also be further developed to support the comparison of pest control practices across countries and to feed into EU policy making and private sector initiatives. Given the potential impact of indicators on decision making, we envision that their development should ideally have a joined character focussing on the harmonisation of the indicator approaches. We highly welcome to discuss with other initiatives how to join forces in the further shaping of these type of farmer targeted indicators.

2.02.P-We096 Towards Balanced Solutions for Considering Safety and Sustainability Trade Offs at an Organisational Scale: The SUNRISE Solution

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Safety and sustainability trade-offs often occur in complex organisational activities such as implementing Safe and Sustainable by Design (SSbD) innovation processes. These are conflicts which emerge when improving one goal comes at the expense of the other. For example, safety may often require short-term resource use that can conflict with long-term sustainability goals. Such trade-offs are unavoidable and demand a sound balanced approach which can help solve conflicts when for example a product designed for safety uses more resources, and/or when prioritising one sustainability goal can negatively impact other. To develop a sound solution for this problem, we are informed by survey and interview methods to determine the factors affecting and influencing potential safety and sustainability trade-offs decision makers face. An analysis of relevant biases is performed to define cognitive defaults for various stakeholder groups (eg. industry, regulators, consultants, academia). The analysis of these defaults explains the underlying reasons why the stakeholders may choose between e.g., products that are potentially damaging but less expensive or more profitable. The analysis of cognitive biases is combined with discursive methods (structured dialogue), which is applied during the interviews phase of the study to stir reflections about social desirability and to systematically collect information about the perspectives, arguments and values of the engaged groups. Gender considerations in decision making processes are also examined in the study. The results of the elicited safety and sustainability trade-offs for the targeted stakeholders

will be used as a basis for defining weights for specific assessment criteria/indicators and impacts in the Horizon Europe SUNRISE project integrated approaches for impact assessment (www.sunrise-horizon.eu) of advanced materials and its digital e-infrastructure thereby presenting one of the first industry oriented balanced solution able to account for safety-sustainability trade-offs in complex decision making.

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2.02.P-We097 Development of a Social Life Cycle Impact Assessment Method Considering the Effects of Child Labour on Education

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Although the global prevalence of child labour has shown a declining trend, approximately 140 million children remain at risk as of 2024. Sub-Saharan Africa accounts for over 60% of global cases, indicating persistent and severe conditions in the region (ILO, 2025). Conventional Social Life Cycle Assessment (S-LCA) approaches have primarily relied on qualitative indicators—such as occurrence frequency or country-level risk categories—to evaluate child labour. While useful for hotspot identification, these indicators have limited capability to support quantitative impact assessment or cross-supply chain comparison.

In this study, child labour is conceptualized as both a loss of educational opportunity and an increase in labour supply. Each aspect is monetized to evaluate the resulting change in human capital. This methodological design is grounded in the Impact Pathway Approach. The analysis targets child labour in the mineral extraction and crop production sectors across 140 countries covered by the GTAP database. Data sources include sector-specific child labour risk information from the Social Hotspots Database (SHDB), integrated with educational and labour statistics published by international organizations such as the ILO and UNESCO.

Specifically, labour hours are treated as the elementary inventory variable and are converted into midpoint indicators through existing risk factors and weighting schemes. A state-transition model capturing stakeholder behaviour and causal relationships is then applied to translate midpoint results into endpoint indicators expressed in life-year units. Finally, changes in human capital are estimated in monetary terms based on the Inclusive Wealth framework, integrating both educational loss and increased labour supply effects.

The study introduces a quantitative method that monetizes the impacts of child labour through changes in human capital, and in extending the assessment scope to 140 countries to enable international comparison using risk factors. This work expands practical applications of the Type II (Impact Pathway) approach within S-LCA and proposes a new framework for quantitative social impact assessment.

The results can be applied to quantify human capital changes attributable to child labour throughout entire supply chains, contributing to corporate and policy-level decision-making for social sustainability.

2.03 Ecological Context of Pollinator Risk Assessment: Challenges Becoming Threats?!

2.03.T-01 A step forward - Comparative tool evaluation under EFSA's 2023 Bee Guidance Document

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Pollinator protection is a critical component of sustainable agriculture and regulatory ecotoxicology. The EFSA Bee Guidance Document (2023) intends to introduce a harmonized framework for assessing risks to pollinators, emphasizing a tiered risk assessment with consideration of time-reinforced toxicity (TRT), mixture toxicity and sublethal effects. Due to its complexity and to simplify the usage, an online, browser-based calculator tool (B-

risk, beta version 1.0.0, first public version, published Nov. 2024) was developed in R by EFSA and published on the EFSA R4U platform.

After publication of EFSA 2023, the authors, on behalf of the CropLife Europe (CLE) bee sub-group, independently started the development of a calculation tool using R (Beexp). The aim was to facilitate a better understanding of the new bee GD and to demonstrate whether the calculations required under EFSA 2023 can be transparently reproduced and applied in the same way as intended by EFSA. Beexp provides all functionalities for screening and tier I risk assessments, assessments of TRT and mixture toxicity. Further functionalities are still in development.

We compared the outcome of the same risk assessment calculations of both tools to get an overview of possible differences. These comparisons highlight potential uncertainties in EFSA 2023 which if left open to interpretation may lead to inconsistencies in the application and evaluation of the new guidance.

It should be emphasized that Beexp is not intended to replace B-risk but rather was developed to verify the B-risk calculations in the absence of a published R code, and ensure consistent interpretation of EFSA 2023.

Beexp also adds a different approach to the workflow outlined by EFSA 2023. In its current state, the functionality of EFSA's B-risk is limited to scenarios fully aligned with the guidance data requirements.

However, in practice screening assessments often involve incomplete datasets, where flexibility is essential to support development of robust testing strategies. Beexp closes this gap by applying EFSA principles while offering enhanced adaptability for screening and higher-tier refinements. The tool enables risk characterization even if data does not fully meet EFSA's input requirements, thereby supporting early decision-making and iterative refinement.

The presentation will illustrate the tool's functionalities, provide the results of the comparison and outline further steps of development.

Disclaimer/Disclosure:

The work was funded by CropLife Europe (CLE) bee group which is a European organization which represents the crop protection sector. Their members are global companies, small and medium-size enterprises (SMEs) and national associations operating in conventional pesticides, biopesticides, plant biotechnology, and digital and precision agriculture.

2.03.T-02 Effects of simultaneous exposure through different routes

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In real life most insects will be exposed to pesticides through simultaneous exposure by different exposure routes. Exposure through contact with sprayed plant material, in combination with exposure through contaminated food, like plant material, pollen and/or nectar will be the general rule and not the exception for most species. Despite this, experimental data on the effect of different simultaneous exposure routes with a single compound or different compounds could not be found.

To get a better fundamental understanding, dedicated experiments were carried out with single exposure through food, through contact and a simultaneous exposure through both routes. The experiments were carried out with honeybees exposed to imidacloprid. Since we know that exposure concentrations in acute tests are not constant the data are interpreted with the BeeGUTS model, which is a Toxicokinetic Toxicodynamic (TKTD) model, that allows for an effects analysis with a time-dependent exposure and can take into account the specifics of a bee. The experiments were carried out with additional observations on effects at intermediate points in time to facilitate the analysis with a TKTD modelling approach.

It showed that the effect of the combined exposure could accurately be predicted for the different doses and timepoints by adding the different (time-dependent) doses, or by multiplying the survival probability of the independent tests.

2.03.T-03 Mapping Honeybee Colony Vulnerability: Integrating Landscape, Exposure, and Effect Modelling for Pollinator Health

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Pollinators are essential for ecosystem services, yet current risk assessment frameworks often rely on simplified scenarios that fail to capture the spatial heterogeneity of real-world landscapes. A major challenge in regulatory decision-making is understanding how specific landscape structures and hive locations drive local risks. To address this, we utilized the xPollinator model to generate vulnerability maps that integrate landscape composition, resource availability, and pesticide exposure profiles.

In this study, we applied the model to apple-producing regions in France to assess honeybee colony health under pesticide use patterns. The resulting vulnerability maps reveal distinct spatiotemporal variations in colony size, demonstrating that hive placement can be a critical determinant of colony survival. We observed that colonies situated near apple orchards faced lower survival probabilities, while those in diverse landscape contexts exhibited greater resilience.

These findings illustrate the potential of vulnerability maps to synthesize complex simulation data into interpretable spatial formats. Rather than offering a standalone solution, the study demonstrates how spatiotemporally explicit methods can support the exploration of high-risk zones and mitigation concepts. This approach aligns with ongoing efforts to integrate landscape-level analyses into pollinator risk assessment, offering a flexible way to visualize how landscape context might inform future scenario development.

Disclaimer/Disclosure:

xPollinator is open-source. This work was partly supported by Bayer AG

2.03.T-04 Modulation of the Effects of local Pesticide Use by Landscape elements on Wild Pollinators in an Agricultural plain

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Wild insect pollinators play a crucial role in maintaining biodiversity and supporting agricultural ecosystems by ensuring the reproduction of flowering plants, yet their populations are declining worldwide. Among the main drivers of this decline are the intensive use of pesticides and the loss of habitats and floral resources. Certain landscape elements such as grasslands can reduce the negative effects of pesticides on wild bees. However the extent to which landscape composition can mitigate or amplify the effect of pesticides on wild pollinators remains largely unknown. The study aims to fill this gap by assessing how landscape elements affect the impact of pesticides on wild pollinator communities (hymenoptera, coleoptera, diptera, and lepidoptera). Over a five-year period, surveys of wild pollinators were conducted in oilseed rape (*Brassica napus*) fields in the Long-Term Socio-Ecological Research (LTSER) Zone Atelier Plaine & Val de Sèvre using two complementary sampling methods: pan traps and sweep nets. Detailed information on pesticide applications (molecules and dosage) was available through farmers' surveys and was used to quantify local pesticide pressure. Pesticides (insecticides, fungicides, herbicides) were modelled using risk-based variables such as Hazard Quotients (HQ) and diversity metrics based on the number of modes of action. Their effects on pollinator abundance, species richness, and diversity were analysed together with the percentages of grassland, woody elements, and organic farming within a 500 m radius. Interactions between landscape variables and pesticides were included to determine how landscape composition modifies pesticide effects. The siland method from Carpenter and Martin was applied to the same models to estimate simultaneously the distance and the effect of landscape variable without any prior assumption on the spatial scales of influence and therefore explore the hypothesis that the landscape variables can affect pesticide effects at different scales. Species richness and diversity were negatively influenced by insecticide and herbicide use whereas the presence of grassland increases pollinator abundance, while forest cover enhances species richness. The study highlights that these landscape elements were shown to mitigate pesticide-risk effects at different spatial scales on wild pollinators. These results emphasize the need to consider multiple distances when assessing their impact on pollinator diversity.

2.03.T-05 Modelling Population-Resource Relationships Using a Spatially Explicit SolBeePop

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Solitary bees are important members of wild pollinator communities, contributing to agroecosystem biodiversity and the pollination of crops and wild plants. The life cycle of univoltine solitary bees involves a short post-emergence activity period, during which females independently provision successive brood cells. Reproductive output is sensitive to landscape-level resource availability, but the relative roles of nesting and floral resources

in shaping natural population dynamics remain poorly resolved. Ecological models are essential tools for understanding and predicting context-dependent population responses to support land management strategies and ecological risk assessment. By synthesising available data and information on species ecology, models can also identify key knowledge gaps to guide empirical research and future model refinement. SolBeePop is an existing trait-based, spatially implicit population model for several species of solitary bee. Here, we extend SolBeePop to a spatially explicit framework incorporating both nesting and floral resources to predict relative population dynamics across heterogeneous landscapes. We characterise nesting and floral resources for six landscapes and test alternative implementations of resource selection, density dependence and dispersal for realistic simulation of spatially structured populations. Assessment of model versions will involve expert judgement and qualitative comparison of model outputs (e.g. carrying capacity, mean brood cells per female, proportion of nesting patches occupied) to empirical population patterns. Analysis of model outputs in relation to landscape metrics will reveal how population outcomes vary across a range of resource availability scenarios. The spatially explicit SolBeePop framework provides a foundation for future investigations into how natural population dynamics respond to the interactive effects of landscape composition and configuration, changing floral phenology, agricultural management practices and other stressors in agricultural landscapes. In addition, we demonstrate a systematic approach to developing and testing alternative model structures using multiple information sources when empirical data is lacking.

Disclaimer/Disclosure:

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2.03.P Ecological Context of Pollinator Risk Assessment: Challenges Becoming Threats?!

2.03.P-We098 The Revised EFSA bee Guidance (2023) Three Years on: An Industry Stakeholder's Perspective and Recommendations

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In 2024, Crop Life Europe (CLE) conducted a comprehensive impact analysis of the revised EFSA 2023 Bee Guidance Document. The assessment evaluated: 113 active substances/products (23 insecticides, 43 fungicides and 47 herbicides) based on 197 different uses (GAPs).

As an outcome of this analysis, CLE categorised elements of the EFSA 2023 Bee Guidance Document into four groups:

- Category 1: Ready for implementation in the short-term (<2 years)
- Category 2: Can be implemented in the medium term (within 3 years)
- Category 3: Further development required before implementation (>3 years)
- Category 4: Additional topics that would benefit from a joint industry, regulator and academia workshop

Our impact analysis of the revised EFSA Bee Guidance Document (2023) identifies several elements suitable for short-term implementation, including honey bee screening and Tier I risk assessment of predicted individual effects (PIE), exposure estimates for during-flowering contamination, and the application of dose-response models with extrapolation factors for surrogate LD50 values. In the medium-term, pre-flowering factors (PFF) are a welcomed addition, but require additional calibration aligned with BBCH growth stages, while the time-reinforced toxicity assessments (TRT) face practical hurdles meeting the ~10% mortality threshold at 100 µg/bee/day, even for substances of low toxicity. In the long-term, several aspects require further development, particularly the establishment of robust protection goals for non-apis species, the determination of reliable toxicity extrapolation factors (TEFs), and harmonised analysis and interpretation of non-Apis higher tier studies. Finally, our analysis identifies topics that would benefit from a joint industry, regulator and academia workshop assessment such as experience with the EFSA Bee Calculator Tool and the assessment of higher tier bee studies.

CropLife Europe recommends a stepwise implementation plan (roadmap), whereby some elements of the lower tier risk assessment for honey bees may be workable in the short-term with improvements to the EFSA Bee calculator tool. Other aspects of the guidance require further development prior to their implementation.

2.03.P-We099 Towards Harmonised First-Tier Pre-Flowering Factors in the European Food Safety Authority 2023 Bee Guidance: Linking BBCH Growth Stages to Application Timing

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The European Food Safety Authority's revised guidance on the risk assessment of plant protection products for bees (EFSA, 2023) introduces a new parameter – the pre-flowering factor (PFF) – to estimate dietary exposure in lower-tier assessments. The PFF extrapolates residue concentrations in pollen and nectar from applications made during flowering to those made before flowering, based on the number of days between application and crop flowering onset. A key challenge is that this time interval is not typically specified on product labels or Good Agricultural Practice (GAP) descriptions, as it varies by region and season. In contrast, the crop growth stage (BBCH) at application is consistently reported. To address this, we propose using the AppDate model, already employed in environmental exposure assessments, to estimate application dates for crop BBCH stages 10–59 and the onset of flowering (BBCH 60). This enables calculation of the minimum interval between pre-flowering application and flowering, and thus the appropriate PFF. We present initial analyses for several bee-attractive crops to illustrate the approach. Our findings support the development of default first-tier PFF values for major crop groups, which could be agreed upon by regulatory authorities ahead of implementing the EFSA 2023 bee risk assessment framework.

2.03.P-We100 The Equivalence Testing Approach for the Statistical Analysis of Higher Tier Pollinator Studies: Recommendations and Challenges

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The 2023 revised European Food Safety Authority (EFSA) bee guidance mandates a shift from difference to equivalence testing for higher-tier pollinator studies, creating significant new challenges for study design and the overall evaluation and interpretation of data for regulatory purposes. The core question is determining whether this new statistical framework is practically feasible, particularly concerning the number of replicates needed to demonstrate the equivalence. To address this, we evaluated the performance of three statistical models (t-test, two-way ANOVA, and a linear mixed-effects model with an autoregressive structure) using a comprehensive simulation study. We investigated the number of replicates required to achieve 80% statistical power to conclude equivalence under various scenarios, including different true effect sizes, background variances, and temporal correlations.

While it could be demonstrated that one model fits best for purpose in terms of efficiency and statistical power, the number of replicates required in all models to demonstrate robust and reliable equivalence is substantially greater than recommended in current test guidance. Even in a best-case scenario, demonstrating equivalence for a product with no true effect (0%) required 7 replicates, while a product with a 9% effect required over 600. We also found that reducing initial colony variability as one aspect of potential improvement had only a minor impact on the required number of replicates.

These results indicate that the number of replicates demanded by the equivalence testing approach may be unmanageable in typical semi-field study designs used for regulatory testing. Our work highlights a critical feasibility gap and urges caution in the regulatory implementation of this approach until it is clear that studies can be realistically designed and conducted to meet the statistical requirements for demonstrating acceptable risk.

2.03.P-We101 From Controlled Tests to More Realistic Exposure: Advancing Risk Assessment Tools for Solitary Bees Using Osmia Species

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Solitary bees such as *Osmia cornuta* and *Osmia bicornis* are increasingly recognised as relevant non-Apis pollinators for pesticide risk assessment. As global regulatory guidance moves toward broader pollinator protection, validated and operational methods reflecting solitary bee biology are still needed for consistent implementation across laboratories and semi-field facilities.

These protocols support ongoing guideline development by evaluating and improving two complementary

methodological approaches. In the laboratory, we implemented a chronic oral toxicity test following a draft guideline using a toxic reference (dimethoate) to verify test feasibility and stability of exposure conditions. The method yielded clear and repeatable dose-dependent effects for *O. bicornis*. For the second species, *O. cornuta*, expected toxicity patterns were also observed, although increased variability in survival and feeding behaviour indicates areas requiring procedural adjustments before routine regulatory use.

At the semi-field level, we contributed to an international ring-test assessing a tunnel-based protocol for *O. cornuta*. This design enabled the controlled evaluation of exposure and effects across the first generation, including metrics related to reproduction, nesting activity and offspring production. The ring-test demonstrated that the protocol is feasible and transferable across multiple facilities and captures endpoints relevant for future guidance. Based on our findings and conclusions, the determination and pertinence of the exposure period will be discussed.

Overall, these results advance the regulatory readiness of solitary bee testing by identifying robust methodological elements, highlighting species-specific considerations and informing the refinement of future guidance. Integrating these methods into risk assessment frameworks will support a more comprehensive and ecologically relevant evaluations of pesticide impacts on pollinator communities.

2.03.P-We102 Pesticide Risk to Pollinators in Agricultural Landscapes: Comparison of *Apis mellifera* and *Osmia bicornis* in Two Cropping Systems

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Arthropod populations, including key pollinators, are declining worldwide due to human-driven factors such as land-use change, intensive agriculture, and pesticide use. Understanding pesticide exposure in both managed and wild bees is crucial for safeguarding pollinator health and crop productivity. This study assessed pesticide residues in pollen collected from *Apis mellifera* hives and *Osmia bicornis* nests at agricultural sites with apple orchards or oilseed rape fields, varying in the proportion of semi-natural areas (1.7-18%; e.g., forest, extensively managed permanent grasslands, field borders) within a 1 km radius, with a bee hive or nest as the center points. Pollen from *A. mellifera* was sampled using pollen traps active for one day in spring at the peak of crop flowering (13th April for orchards and 30th April for oilseed rape) and in summer after crop flowering had ended (18th July for orchards and 27th June for oilseed rape). Pollen from *O. bicornis* nests was sampled at the end of their foraging season, representing thus pollen collected by the bees throughout the season, from early April to the end of May.

Among 120 pesticides analysed in pollen of both species, 48 were detected, including some banned in the EU (e.g., chlorpyrifos, carbendazim, thiophanate-methyl). Insecticide acetamiprid was found in all samples, and fungicides azoxystrobin, difenoconazole, fluopyram, tebuconazole, and trifloxystrobin were detected in at least 75% samples. Pesticide Risk Index (PRI) for pollen from each bee hive per season and PRI for each *O. bicornis* nest were calculated as the sum of the ratios of each detected pesticide concentration to its oral 48h LD50 for *A. mellifera*, to capture the combined hazard and exposure level to multiple substances. For all sites but one, PRI for *A. mellifera* was higher in spring, and for most sites PRI was higher for *A. mellifera*. PRI for *A. mellifera* calculated for spring or summer did not correlate with the PRI for *Osmia* (?0.4, Pearson correlation). Relations between the species-specific PRI and risk calculated based on crop structure and associated pesticide use will be discussed. We will also explore the potential mitigation effect of semi-natural areas in reducing exposure of bees to pesticides.

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2.03.P-We103 Solitary bee (*Osmia bicornis*) larvae test- Testing different pollen sources

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In May 2023, EFSA published its revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). This updated the data requirements to include acute, chronic and larvae testing for all three groups. To date, some research has developed methods for feeding *Osmia bicornis* larvae under laboratory conditions (i.e. Sgolastra et al. 2015, Exeler et al. 2019), which can be used to evaluate the effects of plant protection products on the larvae. In this study, three different pollen sources were tested to evaluate their suitability as food for the developing larvae. The findings demonstrated that both pollen collected and provided for eggs by the parent *Osmia bicornis* and pollen collected by honeybees were suitable for larval development to pupation, but not pollen collected directly from oak tree (*Quercus* spp.).

2.03.P-We104 Pollinator Acute Contact Toxicity Tests in Overspray Design

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For some plant protection products applied by spraying, currently ongoing evaluations on EFSA- and member state level point out the need for a more realistic acute contact risk assessment of pollinator species by direct spraying or spray-drift exposure. While there are established standard protocols available to quantitatively address such risks (OECD 214, OECD 246) in general, no methodologies or basic technical guidance is agreed to capture the acute contact risk by the overspray route.

We herein present first investigations of our developed experimental setup to address exposure in an overspray scenario on laboratory scale. Handling of honey bees and bumble bees as well technical details on study conduct are discussed. Sensitivity of such an experimental setup is demonstrated by assessing the 24- and 48-hrs LC50 values of honey bees and bumble bees exposed to Dimethoate compared to OECD 214 and OECD 246 reference values, intra-laboratory historical control data as well as commonly used rates in semi-field trials' positive controls (e.g., OECD 75).

Our procedure allows to address the data request by some competent authorities even in the absence of an agreed guideline or testing protocol in an excellent scientific and regulatory-compliant way. Such a study design may serve as basis to fulfil the demand of additional exposure scenarios in the risk assessment.

2.03.P-We105 The Effects of Plant Protection Product in Chronic Oral Toxicity Test on Honeybees

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Plant protection products (PPPs) are only authorised for marketing after a thorough environmental risk assessment. The assessment requires a series of ecotoxicological tests on non-target organisms, including honeybees (*Apis mellifera* L.). OECD Guideline No. 245 introduces a chronic oral toxicity test on adult worker honeybees under laboratory conditions over an exposure period of 10 days. In the study, PPP with the active substance pyriproxyfen was used as the test item. Pyriproxyfen is a pesticide and insect growth regulator. Chronic exposure to bees of pyriproxyfen can reduce survival rates and impair behaviors critical for colony health. The study involves feeding bees a diet containing the test item for 10 days and monitoring sublethal effects, mortality and food consumption. Freshly emerged young worker bees are collected for testing. For a period of 10 days, bees are fed a 50% aqueous sucrose solution treated with the test item with ad libitum access. Every day, the feeders are removed, weighed, and replaced with new ones containing a fresh diet. The insects are observed for mortality and other behavioural effects (i.e. apathy, movement coordination problems) at daily intervals up to 10 days of exposure. The test lasts 10 days. Weights of the feeders are recorded every 24 hours (until the end of the test). On the basis of the weight differences after 24 h, the amount of the consumed treated diet is determined. In addition to the group exposed to the test item, a control group and a reference item group participate in the test in order to verify the precision of the test procedure and the sensitivity of the biological test system. The aims of the study is to determine the chronic oral toxicity of the test item to honeybees and to demonstrate that the median lethal concentration and median lethal dietary dose are higher than the test item concentration used for exposure. The test takes into account chronic exposure scenarios that are representative of real-life conditions where residues persist in bee food supplies or crops.

2.03.P-We106 Bumblebee (*Bombus* spp.) larvae test- Approaches and first achievements

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In May 2023, EFSA published its revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). This updated the data requirements to include acute, chronic

and larvae testing for all three groups. To date, very little research has focused on artificial feeding of bumblebee larvae under laboratory conditions—a critical first step toward developing a standardized bumblebee larval test. Based on the available literature, efforts were made to establish a practical feeding regime that supports proper larval development while remaining feasible for routine laboratory work. First attempts at food compositions, amounts fed, daily feeding times, plates used to raise the larvae, and grafting techniques are described for our lab.

Although more trials would need to be done to confirm the findings, first results suggest that synchronization is most easily achieved by collecting egg packages from colonies and allowing them to hatch in a Petri dish. The eggs need to be collected from hives that are not too old, held at appropriate temperatures and are in an overall good state. Once synchronized, larvae are transferred to rearing plates and fed increasing amounts of two different artificial diets according to their age. If the overall amount of food given was too little, larvae did not have enough energy to pupate. If the overall amount of food was too much, larvae died from overfeeding/molding. Young larvae (1st and 2nd instars) survived best when fed once per day with daily increasing amounts not exceeding 8 µL baby diet/day. Older larvae (3rd and 4th instars) survived best when fed twice per day, with amounts of about 5-10 µL of the second feeding solution/feeding. Feeding should be stopped after 18 days or when first signs of spinning are noticed. The only hatching success was achieved in larvae which were transferred to fresh cups at the first signs of spinning (after defecation) and no further manipulation took place.

These first attempts provide a foundation for future development of a standardized guideline for bumblebee larval testing.

2.03.P-We107 Sensitivity difference between Summer and Winter honeybees

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As bees in general and honeybees specifically are important for pollination of crops, the potential impact of plant protection products and other toxicants on this group has been investigated extensively. However the biology of a honeybee biology differs throughout the season. With the so-called winter bees having a longer lifespan (months) then summer bees (weeks). As a result, there has been some debate on whether summer bees and winter bees differ in their sensitivity towards pesticides.

Tests were conducted with Summer and Winter honeybees exposed to imidacloprid in an acute contact test. The tests were carried out according the OECD guideline for acute contact exposure. The data were interpreted with the BeeGUTS model, this is a Toxicokinetic Toxicodynamic (TKTD) approach based on the GUTS modelling framework, but with some adaptations made in the exposure part to take the specifics of a bee into account.

The data showed that winter bees are about a factor of seven more sensitive than summer bees. But the 48 hour LD50 values are more or less identical. The higher overall sensitivity of the winter bees is caused by the slower kinetics, so effects take longer to develop. This is not reflected in the 48 hr LD50 values but the 96 hr LD50s are already substantially lower for the winter bees. This again implies that comparing sensitivity based on LD50 values, without taking the development of effects over time into account may lead to false conclusions about the sensitivity of an exposed species.

2.03.P-We108 BeeGUTS Meets Joint Survival: Multi-Species Toxicant Response Modelling; Integrating beeGUTS and multiGUTS for Ecotoxicological Risk Assessment

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Mechanistic effect modelling has become a vital approach for understanding ecotoxicological impacts, explicitly accounting for uncertainties and variabilities in environmental conditions and processes.

In this study, we combined two recently published TKTD approaches: the joint survival modelling framework for multiple species (multiGUTS; Singer et al. 2022) and the bee-specific BeeGUTS model (Baas et al. 2022). For the test substance Dimethoate, acute contact and acute oral studies were available for five bee species: the honeybee (*Apis mellifera*), bumblebee (*Bombus terrestris*), leafcutter bee (*Megachile rotundata*) and two mason bee species (i.e. *Osmia bicornis* and *O. cornuta*).

The objectives of this study were to evaluate whether the multiGUTS approach, previously applied to aquatic species, is also applicable to bees, and to assess whether oral toxicity can be predicted from acute contact tests.

Current results clearly indicate that the multiGUTS approach is suitable for Dimethoate and the tested bee species, and that it may enhance GUTS modelling for datasets with limited data points and enabling the prediction of mortality under untested exposure scenarios across species.

2.03.P-We109 BeeGUTSmet: Extending TKTD Modelling for Honey Bees by Integrating Metabolism and Bioaccumulation

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Toxicokinetic–toxicodynamic (TKTD) models are increasingly used in environmental risk assessment of insecticides for honey bees. The BeeGUTS model, based on the GUTS-RED framework, integrates acute and chronic toxicity data using a simplified structure that combines uptake, elimination, and damage into a single rate constant. While effective for many substances, this simplification assumes rapid equilibrium between external exposure and internal damage, which may not hold for compounds with distinct metabolic or bioaccumulative properties.

To address this limitation, we developed BeeGUTSmet, an extension that explicitly models internal toxicokinetics using experimentally derived metabolic half-lives. These values were obtained through in vivo dynamic Total Compound Balance (dTCB) assays, allowing us to simulate internal concentrations over time and separate toxicokinetic from toxicodynamic processes. This mechanistic separation enhances biological realism and enables more accurate predictions, particularly under chronic exposure conditions.

BeeGUTSmet was calibrated and validated using insecticides with a range of kinetic profiles—from rapidly metabolized to highly persistent. The model consistently improved predictions, where delayed mortality or bioaccumulation played a significant role. By integrating metabolism explicitly, BeeGUTSmet enhances the biological realism of TKTD modelling and strengthens its applicability across diverse exposure scenarios. Importantly, BeeGUTSmet complements the original BeeGUTS model rather than replacing it. By maintaining compatibility with existing regulatory frameworks while expanding applicability to substances where internal kinetics are critical, BeeGUTSmet represents a meaningful advancement in TKTD modelling for pollinator risk assessment. Its integration of metabolism as a central driver of internal dose supports more robust and reliable evaluations of long-term pesticide effects.

2.03.P-We110 BeeGUTSmet: Technical Implementation of an Extended TKTD Model for Honey Bees

David Schneider, Vanessa Roeben, Marion Zaworra, Kathrin Vogelsang, Kristin Raschke, Heika Sobczyk, Kai Becker and Thomas G. Preuss, Bayer AG CropScience, Germany

BeeGUTS is a toxicokinetic–toxicodynamic (TKTD) model developed for honey bee (*Apis mellifera*) risk assessment. It is based on the reduced General Unified Threshold Model of Survival (GUTS-RED) and provides a practical framework for integrating acute and chronic toxicity data. This approach has proven valuable for regulatory applications, offering a streamlined representation of uptake, elimination, and damage accrual through a single dominant rate constant.

However, certain compounds exhibit kinetic profiles that challenge this simplification, such as rapid metabolism or bioaccumulation. To address these cases, we developed BeeGUTSmet, an extended TKTD model that explicitly separates toxicokinetics from toxicodynamics. Internal concentrations are calculated using a physiologically structured approach with substance-specific elimination parameters, enabling metabolism to be represented mechanistically rather than implicitly.

In this poster, we highlight the technical implementation of BeeGUTSmet in detail, including its modular design for uptake and internal processing, and demonstrate how these refinements improve the ability to capture delayed effects and time-dependent mortality patterns under chronic exposure scenarios. Comparative evaluations indicate that this approach enhances predictive robustness across diverse exposure assumptions, supporting more reliable extrapolation beyond standard laboratory conditions.

By combining mechanistic accuracy with regulatory relevance, BeeGUTSmet strengthens the TKTD modeling toolbox for pollinator risk assessment and provides a pathway toward dynamic, scenario-based evaluations that reflect realistic exposure patterns.

2.03.P-We111 Development of Chemical and Crop Specific Honeybee GUTS Exposure Scenarios for Assessment of Time-reinforced Toxicity

Thomas Sinclair¹, Christof Schneider¹ and Pernille Thorbek², (1)BASF, Germany, (2)BASF, United Kingdom

Honeybee health is a critical component of sustainable agriculture, and regulatory frameworks increasingly require robust risk assessments for plant protection products. The EFSA 2023 Bee Guidance Document requires the assessment of Time Reinforced Toxicity (TRT) for different bee scenarios, which can be assessed using GUTS TKTD modelling. While the bee GD allows for the use of GUTS TKTD modelling to refine risk, it is not clear from the GD what refinement scenarios for risk assessment should be.

Here, we show how the EFSA bee GD methods for estimating exposure can be translated into exposure time series for use in GUTS modelling. This is demonstrated for annual and perennial crops as well as for active period (summer) and overwintering bees using a hypothetical compound.

The proposed methodology for developing exposure scenarios incorporates crop and chemical information including degradation, (multiple) application timings and methods, and residue dynamics in different matrices from a realistic hypothetical compound. The different bee scenarios consume different amounts of nectar and pollen, and we show how this can be parameterised.

Finally, a hypothetical but realistic case study shows how the parameterised GUTS model together with exposure profiles can be used to calculate margin of safety for refinement of risk.

2.03.P-We112 A Scenario-Based Analysis Using the Model SolBeePop: Exploring Impacts of Foraging Activity, Floral Composition, and Pesticide Exposure in Agroecosystems on Simulated Abundances of Solitary Bees

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Agriculture is essential for human food security, but it also brings negative side effects such as increased homogenization of agroecosystems and in parts intensive pesticide use. This can not only affect human well-being but is under discussion as one reason of drastic declines in many pollinator species. One particularly affected and still under-researched pollinator group are solitary bees. Individual-based models are a promising tool to estimate population-level effects of landscape composition and pesticide exposure. In this study, the model SolBeePop_ecotox was used and expanded to include environmentally relevant factors influencing bee foraging behavior, focusing on two bee species, *Osmia bicornis* and *Megachile rotundata*. Environmental scenarios were developed based on real weather data and diverse floral resource availabilities representing treated crop cultures and retreat areas of natural plant species, i.e., flower strips. Those were combined with annually repeated applications of the insecticide dimethoate. The key metric for effect evaluation was the exposure level at which the simulated populations declined by 50% at six different time points within the simulated period of 15 years. Explicitly, the proportion of how many bees are foraging was derived from prevailing weather conditions, with the result that unsuitable weather in contrast to suitable conditions leads to decreased population survival but increased resilience towards pesticide exposure. Furthermore, the results show that pesticide-free flower strips generally improve the survival rate of simulated bee populations and, in particular, can increase their resilience against pesticide use, especially at high pesticide concentrations and in low-resource agricultural systems. Contrarily, mass-flowering crops and high-diversity polycultures already provide near-optimal foraging conditions and showed reduced effects when coupled with flower strips. Momentarily, the developed scenarios are based on an exploratory approach and future studies should confirm these findings by using real landscape structures and observed solitary bee abundances. This current work gives important insights on how to depict agricultural resources in an implicit way, further enhancing individual-based model understanding. Eventually, research like this will be fundamental for evaluating the protection of solitary bees.

2.03.P-We113 xPollinator – A Flexible Landscape Modelling Framework for the Assessment of Land Use and Pesticide Impacts on Pollinators

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Demands for more holistic regulatory ecological risk assessment and risk management for plant protection products increasingly require higher-tier tools, such as landscape-scale models, to address real-world spatial and temporal variability in exposure and effects. Such approaches are inherently complex regarding, e.g., environmental conditions, agricultural practices, exposure and environmental fate processes, species ecology, or effects to organisms. Furthermore, landscape-scale models must manage large volumes of (geo-)data and deliver

interpretable results.

The open source xLandscape framework (<https://github.com/xlandscape>, Schad et al. 2024) provides a modular design for integrating such processes, models and data to manage such complexity. Modularity has advantages for collaboration, harmonization, flexibility, consistency, transparency, and validation. With respect to collaboration, scientists developing individual models (e.g., a new effect model) can build on existing modules and data and can thus focus on the problem at hand and domain of expertise.

With xPollinator we introduce a landscape-scale model to assess effects of pesticides on honeybee colonies. xPollinator is composed of land use, bee forage, pesticide use, exposure and effect modules and is based on the xLandscape framework. The BEEHAVEecotox model (Preuss et al. 2022) is integrated as a module to simulate honeybee hive development and effects of pesticide applications. The bee forage module of xPollinator allows to simulate nectar and pollen occurrence in space and time in real-world landscapes. Thus, effects of different land use and landscape structures on colony health can also be studied using a range of agricultural landscape scenarios.

The poster will present the concepts and technical implementation of xPollinator. Results from simulations illustrate its capability to simulate distinct impacts on honeybee colony dynamics (e.g., changes in colony size, honey production, overwintering survival) based on chemical application patterns, and how the model manages data complexity to yield consolidated, relevant endpoints for pollinator risk assessment on the landscape scale. An interactive, web-based interface (BeeView) allows for result visualization. xPollinator will be made public at GitHub.

Disclaimer/Disclosure:

xPollinator is open source. The development was partly supported by Bayer AG.

2.03.P-We114 Buzzing Toward Better Risk Assessment: Advancing Bee Effect Modelling with the ICPPR Bee Modelling Interest Group

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Mechanistic pesticide exposure and effects models have the potential to improve the efficiency and accuracy of environmental risk assessments for bees and other pollinators. Several models have been introduced or are under development, spanning organism-level effects (e.g., toxicokinetic-toxicodynamic models), colony models, and population models, for a range of taxa including honey bees, bumble bees, and non-Apis bees.

However, these models are not yet well integrated into actual risk assessment processes, and a number of challenges remain. In response, the Bee Modelling Interest Group was established within the International Commission for Plant-Pollinator Relationships (ICPPR), with the aim of improving the acceptability and usability of exposure and effect models in bee risk assessments.

The interest group provides a platform for exchange and discussion between stakeholders from different groups including regulators, industry, contract research organizations and academia. Within the interest group, three work groups have been created based on a survey of members' perceptions of the urgency and feasibility of various topics: (1) improving characterization of exposure pathways relevant for bees, (2) incorporation of sublethal effects in honey bee colony models, and (3) establishing a bee model inventory.

We will introduce the aims and activities of the ICPPR Bee Modelling Interest Group and its three active work groups. Through these collaborative efforts, we aim to accelerate the adoption of mechanistic models within regulatory frameworks, ultimately enabling more accurate and efficient pollinator risk assessments that better protect bee populations while supporting sustainable agriculture.

2.03.P-We115 AI-driven Image Analysis for Increased Accuracy and Precision of Honey Bee Colony Health and Productivity in Regulatory Field Studies

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Accurate measurement of honey bee populations and colony health is crucial for ecological research and the regulatory crop protection product studies required by national authorities like the U.S. EPA and EFSA. Traditional visual estimation methods, such as the 'Liebefeld Method', for determining "colony strength" are subjective, leading to inconsistent data, often with a high margin for error. To address this, Corteva Agriscience has developed innovative artificial intelligence systems that replace manual estimation with automated image analysis. One AI-driven system automatically and precisely counts individual honey bees on hive frames,

consistently maintaining over 95% accuracy; an 18% increase in accuracy compared to traditional methods. A complementary tool assesses overall colony organization by tracking the distribution of in-hive resources such as nectar, honey, brood, and pollen. Together, these AI-driven systems provide a more reproducible, accurate, and consistent alternative to traditional methods, significantly reducing subjective bias and human error. These tools are currently undergoing Good Laboratory Practices (GLP) validation, with the goal of full implementation in regulatory semi-field and full-field studies. By providing more reliable and precise data, these systems will enhance the quality of environmental risk assessments on honey bees and support the safe use of crop protection products. This investment in AI-driven technology sets a new standard for pollinator safety research and represents a significant step forward in refining ecotoxicological safety testing, making it faster, more accurate, and more reliable.

2.03.P-We116 Quantifying Landscape-Mediated Bee Exposure Refinement: Deriving Landscape-Specific Refinement Factors (RFs) to Inform Regulatory Risk Assessment, per EFSA 2023 Guidance

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Regulatory risk assessments for Plant Protection Products (PPPs) in Europe often rely on conservative tunnel studies, leading to overestimations of bee exposure in real-world agricultural settings. Building on the 2023 EFSA Guidance on the risk assessment of PPPs on bees, this project addresses the critical need for field data to refine exposure estimates. The main objective is to quantify the dilution effect of agricultural landscape complexity on pesticide residues in honeybee-collected pollen and nectar. The research will employ a multi-site field study designed as a landscape complexity gradient, using worst-case tunnel studies as a crucial control. This setup will allow for the calculation of Landscape-Specific Refinement Factors (RFs). Using GIS analysis, key landscape metrics, such as the percentage of semi-natural habitat, will be identified and ranked as predictors of residue dilution. This work, which continues a successful preliminary study from 2024, aims to generate a first regulatory-relevant dataset for informing risk assessments and validating predictive models like BEEHAVE.

2.03.P-We117 How Pesticide Uses and Agricultural Landscape Interact to Produce a Landscape of Pesticides Affecting Pollinator Communities

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Maintaining pollinator communities across agricultural landscapes is a major issue for wild plant diversity and crop yields. Recent studies have shown a general decline in insect populations, especially in agricultural environments and pollinators are no exception. A major cause of this decline is related to intensification of agricultural practices which consists both of a simplification of the landscape and the widespread use of pesticides, particularly insecticides. Studies of the impact of pesticides on pollinating insects have mainly focused on local effects at field level. However, pollinators are affected by pesticides throughout their life cycle and exploit resources at the landscape level. These landscapes are not homogeneous: they vary in composition and configuration and they can modulate pesticide exposure, in particular by altering pesticide flows and fate. The aim of this project is to understand how landscape affects pollinator communities, while integrating cumulative pesticide uses in order to characterize the pesticide landscapes to which pollinators are actually exposed. To this end, the study focuses on pollinator communities and associated plant communities in permanent grasslands in Brittany. Although these habitats do not directly receive treatments, they are subjected to pesticide flows from the surrounding landscape. Pollinator's exposure is evaluated by quantifying pesticides in soils, floral resources and pollinators themselves, in addition to knowledge of agricultural practices in the surrounding landscape. These data have two main purposes. The first one is to have an overview of pesticide landscape exposures for pollinators collected in permanent grasslands. The second one is to evaluate pesticide contaminations in grasslands and the transfers of pesticides from soil to pollinators through floral resources in these habitats. The results obtained will provide essential information for assessing the risks to these communities in agricultural environments and understanding how the landscape can modulate these risks. They will also make it possible to propose a new scale for assessing pesticide-related risks.

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2.03.P-We118 Beyond Mortality: Preliminary Assessment of Sublethal and Lethal Effects of Technical and Formulated Fungicide on Pollinators

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Pesticides are considered one of the leading causes of honeybee (*A. mellifera*) colony decline. While extensive research regarding the effects of herbicides and insecticides on honeybee has been carried out, the effect of fungicides on honeybees has received less attention, probably because their effects may not be apparent and are more difficult to diagnose due to their generally lower acute toxicity.

One of the main steps in estimating the risks of exposure to fungicides for pollinators is to understand how and to what extent bees are exposed to these active ingredients. Although fungicides are typically not considered acutely toxic to bees, some active ingredients with low toxicity may pose chronic risks to bees. Depending on the application method and purpose, bees may be exposed to fungicides orally, through contact, or by inhalation. Additionally, the composition of the honeybee gut microbiota is closely related to the diet and is therefore simultaneously exposed to fungicides. Therefore, in addition to evaluating the lethal effects in honeybees, it is equally important to assess potential sublethal and indirect effects, including the alteration of pollinators basic gut microbial communities.

In the present case study, we evaluate the effects of a fungicide commonly applied via seed-treatment coatings on pollinators, assessing both the technical and the formulated product. All relevant laboratory exposure routes were considered: acute contact and oral toxicity tests (*A. mellifera* and *Bombus terrestris*), chronic oral exposure (*A. mellifera*), and a repeated-dose larval toxicity test (performed only for the formulated product).

Results revealed differences in responses between the technical and the formulated product. Moreover, sublethal effects occurred only with the formulated product, including intestinal injury as indicated by fecal appearance.

As reported in literature, even in the absence of immediate mortality, exposure to fungicides can still affect bees in subtle but significant ways, such as altering the gut microbiota. For this reason, it would be useful to evaluate the real impact of fungicides on pollinators by introducing microbiota analysis during the laboratory tests and considering the extension of the test durations, at least for chronic oral exposure on honeybee, to assess potential long-term effects on bee health following exposure.

2.03.P-We119 Pollinator Risks of Neonicotinoid Residues in Maize Guttation: Toxicity and Ecological Implications

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This study offers a thorough assessment of the ecotoxicological risks posed to honey bees (*Apis mellifera anatoliaca*) by neonicotinoid seed treatments—specifically thiamethoxam and clothianidin—used in maize (*Zea mays*) farming systems in Türkiye. Considering that honey bees often use guttation droplets as a water source, the research measures both the insecticide residue levels and the acute toxicity from guttation fluid collected from maize plants grown from treated seeds. Controlled bioassays with guttation droplets from thiamethoxam-treated maize showed that bee mortality exceeded 80% within the first four hours of exposure during the initial five days of guttation production. Although mortality decreased afterward, it remained above 50% through day 10. Cumulative mortality assessments at 24 and 48 hours indicated complete bee death by day 9 across all experimental replicates. Interestingly, guttation fluid collected after day 5 continued to cause more than 50% mortality within 48 hours, and droplets taken up to 72 hours caused significant lethal effects. Thiamethoxam exposure led to consistently high mortality (>80%) for nearly all guttation samples collected within the first 72 hours, while clothianidin caused similarly severe outcomes, often resulting in mortality rates between 80–100%. These results highlight the significant risk posed by neonicotinoid seed coatings in maize to pollinator health through guttation-mediated exposure routes and emphasize the urgent need for stronger regulatory oversight and further ecotoxicological research.

2.03.P-We120 Differences in Butterfly Sensitivity to Oral Exposure to Flupyradifurone – A Call for Broader Research on Pollinators

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Agricultural intensification, including widespread use of pesticides, is one of the main drivers of insect biodiversity decline. Currently, regulatory pesticide risk assessment for pollinators relies almost exclusively on toxicity tests on the honey bee (*Apis mellifera*), despite growing evidence that other pollinator species differ substantially in their sensitivity to pesticides. Butterflies, often overlooked in this context, constitute an ecologically important group of pollinators. They may facilitate cross-pollination and access deep floral

structures that are less frequently visited by bees.

This study aimed to determine LD₅₀ values for flupyradifurone administered orally via contaminated sucrose solution to adults of five butterfly species (*Papilio machaon*, *Pieris brassicae*, *Aglaia io*, *Gonepteryx rhamni*, *Polygonia c-album*), allowing for an early comparative assessment of toxicity for this group. The oral 24h LD₅₀s for *A. io*, *G. rhamni*, *P. machaon*, *P. c-album*, and *P. brassicae* were 16.7, 41.6, 53.0, 63.7, and 126.4 µg/individual, respectively. Our results reveal interspecific variation in LD₅₀s, with *A. io* being the most and *P. brassicae* the least sensitive when 24h LD₅₀s were compared, but *P. brassicae* being the most and *P. c-album* the least sensitive when 96h LD₅₀s were compared (3.0 and 41.8 µg/individual, respectively). However, when in the mortality analysis we assumed “dead” to be all individuals that either indeed died or were moribund (unable to walk/relocate, so in fact unable to carry out essential functions), the LD₅₀ values tend to be lower: the 24h LD₅₀ values for *A. io*, *G. rhamni*, *P. machaon*, *P. c-album* and *P. brassicae* were 15.9, 26.2, 8.9, 41.5 and 3.6 µg/individual, respectively, indicating *P. brassicae* being the most and *P. c-album* the least sensitive species (also in terms of their 96h LD₅₀). Whether the order of species sensitivity changes when the LD₅₀ is expressed per unit of mass (µg/g) rather than per individual (µg/individual) will also be discussed. So far, the honey bee appeared more sensitive (24h LD₅₀ of 1.2 µg/individual) than any of the butterfly species tested, suggesting that current risk assessment procedures sufficiently protect a wide range of pollinators. However, the large variability in sensitivity among pollinators indicates that the potential effects of pesticides on this group as a whole are poorly recognized.

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2.03.P-We121 Screening of Pesticides in Pollen Collected by Honey Bees in the Northern Zone

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Pesticides (plant protection products) are used to control pests and support agricultural production, but they can also have negative effects on pollinating insects and their ecosystem services. The primary objectives of this project were to increase knowledge about pesticide exposure of pollinating insects and to contribute to the establishment of a long-term monitoring program for pesticide residues and risks to non-target organisms in the terrestrial environment.

Pollen was collected from honey bee colonies at six locations in Lithuania, Norway and Sweden during May–June 2024. Selected sites represent agricultural areas expected to have high pesticide use. Weekly samples were taken with pollen traps for 3–4 weeks.

At the Swedish University of Agricultural Sciences, pollen samples were analyzed for 173 pesticides using liquid and gas chromatography coupled with mass spectrometry (LC-MS/MS and GC-MS), and 58 of these were detected in at least one sample. An index reflecting the direct pesticide risk to bees was calculated by weighting the measured concentrations of each pesticide with its acute toxicity to honey bees and then summarizing per sample. The insecticide indoxacarb posed the highest risk across all samples, despite it not being approved for use as a plant protection product in the region since 2022. Acetamiprid had the second highest risk and was detected in 96% of the samples. Acetamiprid is one of the few insecticides that is still approved for use in the Northern Zone, with an increase in detection frequency compared to previous studies of pollen in the same region in 2019-2021. The fungicides boscalid, captan, azoxystrobin and fluopyram had the highest maximum concentrations per sample.

At Lund University, the pollen samples were analysed for plant origin using image analysis and machine learning. The commonest pollen type was Brassicaceae, which include cultivated oilseed rape, followed by the Sorbus group, which includes apple. The risk index correlated positively to the proportion of crop pollen in the sample. Oilseed rape and apple were the main flowering crops at the sampled locations and previous studies have identified regions with apple orchards as high-risk areas. In this study, monitoring locations with high

expected pesticide use and potential direct risk to bees were selected, which could be a good strategy when resources are limited.

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2.03.P-We122 Characterising Pollinator Exposure Pathways Through Multi-Matrix Pesticide Residue Analysis

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Understanding co-occurrence of pesticide residues across relevant environmental matrices is essential for characterising pollinator exposure pathways, evaluating exposure modelling, and improving risk assessment. Within the EU Horizon project PollinERA, which aims to advance knowledge on pesticide–pollinator interactions to support European environmental risk assessment and policy, we conducted a coordinated, multi-country residue monitoring campaign to characterise key exposure routes and risks for pollinators. Pollen, nectar, soil, plant material, and surface water were collected in spring and summer 2024 from 18 agricultural sites with either apple orchards or oilseed rape fields in Sweden, Poland, and Italy. Samples were analysed using multi-residue LC-MS/MS and GC-MS methods covering 182 active substances and transformation products. Preliminary results for pollen, nectar, and soil show substantial variation in detections and concentration levels. Across all samples, individual pesticide concentrations ranged from 0.001 to 26,000 ng/g. Non-parametric pairwise tests showed fungicide concentrations were highest in pollen, intermediate in nectar, and lowest in soil (all comparisons significant). Herbicides were highest in pollen (significantly higher than nectar and soil), while insecticide levels did not differ among materials. The most complex mixture occurred in a soil sample collected in spring, containing 72 pesticides (36 fungicides, 18 herbicides, 15 insecticides and 3 metabolites). Twenty-one pesticides were detected in at least 40% of pollen samples, 43 in at least 40% of soil samples, and one compound (insecticide acetamiprid) reached this frequency also in nectar. Overall, soil exhibited the highest detection frequencies, indicating a substantial mixture burden. In contrast, pollen contained higher concentrations, suggesting it may be the more critical exposure route for foraging pollinators, acting both through contact and via feed intake.

These data provide a robust empirical basis for evaluating co-exposure scenarios, identifying realistic pesticide mixtures, and informing the development of PollinERA's pesticide and pollinator co-monitoring scheme. The dataset will support planned pollinator exposure tests, modelling activities within PollinERA, and the development of an integrated, one-system environmental risk assessment approach for pollinators.

Disclaimer/Disclosure:

This research was part of the EU project PollinERA (HORIZON-RIA No. 101135005).

2.04.A Ecological Effects of Chemical Mixtures and Multiple Stressors with Special Emphasis on Space and Time

2.04.A.T-01 Spatio-temporal characterization of pesticide mixtures and associated ecological risks at a landscape scale

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The ecological risk assessment of pesticides is typically performed at the edge-of-field scale and on a substance-by-substance basis. This approach does not adequately capture the complexity of real-world exposure scenarios, where multiple pesticides are applied over one or several crop cycles within heterogeneous agroecological landscapes. In this study, we developed a large-scale modelling framework (ERAHUMED) capable of predicting the spatio-temporal dynamics of pesticide mixtures and associated risks in freshwater ecosystems. The ERAHUMED model was designed to calculate pesticide exposure and ecological risks in the Albufera Natural Park (Valencia, Spain), which is dominated by intensive rice cultivation, an extensive irrigation and drainage network, and the Albufera Lake. Pesticide exposure simulations were performed for eight pesticides currently used in three rice varieties within the park, while pesticide risks were assessed using the multi-substance Potentially Affected Fraction approach (msPAF) for acute and chronic exposure.

The study shows that acute msPAF reached 25% in rice paddies, 22% in ditches, and 7% in the Albufera Lake. The chronic msPAF indicated 55%, 45%, and 32% in rice paddies, ditches, and the lake, respectively. Acute risks were dominated by 1 or 2 compounds, while chronic risks were dominated by mixtures of 3-6 compounds. In early summer, mixtures posing chronic risks were dominated by a combination of herbicides (MCPA, penoxsulam, cyhalofop), while in late summer chronic risks were mainly driven by a combination of these herbicides, an insecticide (acetamiprid) and two fungicides (azoxystrobin, difenoconazole). The southern section of the lake and its adjacent ditches were identified as the areas with the highest acute and chronic exposure and risks.

The implementation of the ERAHUMED model to the ANP shows that aquatic organisms are exposed to both co-occurring and sequential mixtures of pesticides, and that the current substance-by-substance approach used in pesticide risk assessment significantly underestimates ecological risks. During the presentation, we will explain how the ERAHUMED model can support decision-making related to pesticide management, as well as its applicability for conducting landscape-level evaluations for threatened aquatic populations.

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2.04.A.T-02 Toxicity mechanisms and climate govern the global effects of the insecticide imidacloprid on aquatic invertebrates

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The neonicotinoid insecticide imidacloprid is widely used worldwide in terrestrial environments. It is primarily used as a systemic insecticide when applied as a seed coating. After application, it also enters the aquatic environment through spray drift, surface runoff, and leaching. Imidacloprid has been banned for outdoor use in the EU since 2018, and for many uses in the US, but is still widely used elsewhere. This presentation aims to unravel the mechanisms underlying the long-lasting effects of imidacloprid on aquatic invertebrates and how these mechanisms are influenced by temperature, leading to differences in effects across climatic regions. In our studies, we found increased toxicity over time for some invertebrate species, which can be explained by the formation of a toxic metabolite, imidacloprid-olefin, which is poorly eliminated from the body, leading to the absence of an incipient toxicity value. This observation is supported by findings from a long-term population study (120 days) with an insensitive invertebrate species, which yielded extremely low toxicity values (< 0.003 µg/L).

We found, in both single-species and ecosystem-level studies, that increased temperature increases the toxicity of imidacloprid to aquatic invertebrates. This can be explained by an increased uptake of imidacloprid and an increased biotransformation to the toxic metabolite imidacloprid-olefin.

We reviewed 15 mesocosm studies performed with imidacloprid around the world. The reported ranges of nominal NOEC values for mesocosm studies are 0.5–7.5 µg/L, <1–1 µg/L, 0.03–0.3 µg/L, and <0.01–0.1 µg/L for temperate, Mediterranean, subtropical, and tropical regions, respectively. Our review showed that (sub-) tropical communities are more sensitive to imidacloprid than their Mediterranean-temperate counterparts. Temperature differences can partly explain these differences in sensitivity. This indicates the threat posed by pesticides to aquatic ecosystems globally, thereby contributing to global insect decline. It is clear that, besides Europe and the US, the use of imidacloprid should be restricted in other climate regions (especially the (sub-)tropics) as well.

2.04.A.T-03 Seasonality Matters: Opposing Winter and Summer Pathways of Ecological Response to Warming and Pesticides

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Freshwater ecosystems are increasingly confronted with interacting stressors whose impacts can change substantially across seasons. Yet ecotoxicological studies rarely test whether the ecological pathways of stressor effects remain consistent over time. We conducted a full-factorial mesocosm experiment in winter and summer to assess how a mixture of herbicides and fungicides, commonly detected in surface waters, interacts with +4 °C warming to shape community and ecosystem responses.

Pesticides and warming produced complex, non-additive effects from phytoplankton to predatory insects and benthic detritivores, but the dominant pathways shifted seasonally. In winter, the pelagic food web was dominated by top-down control as warming reduced key predators, weakening regulation of grazers, while pesticides suppressed zooplankton. Nutrient stoichiometry (dissolved nitrogen (DN): soluble reactive phosphorus (SRP) ratio) further shaped predator–grazer–phytoplankton interactions, with higher DN:SRP ratios increasing filter-feeder biomass but reducing odonates, thereby altering the trophic cascade. In summer, community dynamics shifted to bottom-up control with warming and elevated DN:SRP ratios associated with increasing phytoplankton, and both warming and pesticides reducing filter feeders, leading to weaker grazing and top-down control.

Benthic detritivores were sensitive to the stressors in both seasons, but consequences differed. In winter, warming and pesticides reduced Asellus and chironomid larvae, yet leaf litter decomposition remained stable. In summer, both stressors reduced detritivore biomass, emergence, and leaf litter breakdown. Across compartments, DN:SRP ratios emerged as a key mediator, structuring grazer, predator, and detritivore responses, highlighting how stoichiometric context interacts with warming and pesticides to shape food-web outcomes

Our findings show that warming can restructure the ecological pathways of chemical mixtures, producing context-dependent, seasonally variable stressor interactions. The shift from top-down mediation in winter to bottom-up mediation in summer highlights the need to integrate seasonal dynamics into empirical assessment and predictive modelling. Ignoring seasonal variability would bias the estimated magnitude and direction of long-term ecological impacts, emphasizing the importance of seasonality for freshwater ecosystem risk assessment under climate change.

2.04.A.T-04 Pulsed Antibiotic Inputs into the Environment May Foster the Spread of Antimicrobial Resistance

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The World Health Organization (WHO) has identified rising antibiotic resistance as a “global threat” underscoring the need to understand resistance dynamics. A key concept is the minimum selective concentration (MSC), the threshold at which resistant bacteria outcompete susceptible ones. While MSC studies usually test single antibiotics, real environments contain fluctuating levels and mixtures from sources like wastewater. This study presents a process-based model of antibiotic accumulation in aquatic systems with and its influence on selection in microbial communities. It incorporates dynamic inputs of antibiotics, photolytic/biotic degradation, cellular uptake and microbial competition. Environmental accumulation from pulses depends on pulse frequency and half-life and can drive selection. Intracellular accumulation depends on intracellular pH, environmental pH, and antibiotic pKa. Antibiotic combinations alter competition depending on synergy, additivity, independence, or antagonism. Synergy can intensify selection even when individual concentrations are below their MSCs. Environmental factors such as nutrients, temperature, and pH modulate but rarely dominate these dynamics. However, intracellular accumulation can strongly affect resistance when conditions are met. These findings clarify how changing concentrations of multiple antibiotics and their chemical properties shape selection and inform mitigation.

2.04.A.T-05 Using Formal Causal Analysis to Differentiate among Chemical, Physical, and Biological Stressors Regarding Observed Environmental Harms

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Causal analysis has a long history of development but in 2005 the U.S. EPA introduced CADDIS (Causal Analysis/Diagnosis Decision Information System) focused on diagnosing causes of stream impairments. We

worked with CADDIS and extended it to a tiered approach designed to diagnose causes of complex environmental harms often involving multiple stressors. The challenge is to identify the relevant stressors, to identify the manner in which they contribute to the observed harms, to ensure that adequate data and evidence are available or can be obtained. A conceptual model is used as a foundation for evaluating the weight of evidence for differentiating among the candidate causes of observed harms. An additional challenge is the consideration of the passage of time in that the original stressor events need to be unearthed through historical reviews. Another time-related challenge is the periodic oscillation in climatic conditions (e.g., cycles of dry and wet periods) that fall within the current longer-term trajectory in climate. This emerged as important in one of our case studies and was tracked using dendrochronology (i.e., tree ring analyses).

We illustrate application of the methodology using three cases which individually highlight evaluations of chemical, biological, and physical stressors. The first case involves differentiating among air pollution, saline groundwater, and plant diseases for determining the main causes of crop and plant damage in the Yemen desert; the second case focuses on biological and chemical stressors contributing to losses of honey bees in the United States; the third case evaluates the role that water management and other factors explain the loss of a highly valued oyster fishery and adverse impacts to wetlands impacts in a major river and estuarine system.

While the methodology serves to identify causes of harm, it also serves to guide solutions. The third case - loss of valued oyster beds - will be used to describe what has emerged as a solution. That solution weaves together educational, sociological, political, and scientific elements.

2.04.B Ecological Effects of Chemical Mixtures and Multiple Stressors with Special Emphasis on Space and Time

2.04.B.T-01 Applying Comprehensive Ecological Risk Assessment to Urban Tropical Rivers: Departures from Temperate River Paradigms

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Understanding contaminant dynamics and ecological effects in tropical urban rivers remains a major challenge, as most ecological risk assessment (ERA) frameworks are derived from temperate regions of the Global North. These frameworks often overlook the distinct climatic, hydrological, and socioeconomic conditions of tropical environments. This study aimed to generate empirical data on multiple pollutant stressors and evaluate their ecological risks across seasons within an urban tropical river basin in Ghana. Water samples were collected from 12 sites along the river continuum during both dry and wet seasons, including adjacent groundwater sources. Analyses covered physical-chemical parameters, historical pollutants (nutrients and heavy metals), and quantification by GC-MS/MS of contaminants of emerging concern (CECs), including pharmaceuticals, personal care products, hormones, and PFAS. Ammonia (range: 0.-21.5 mg/L; Risk Quotient [RQ]=976) and Nitrate (4.4-75.0 mg/L; RQ=66) posed the highest nutrient-related risk, exceeding aquatic life protection limits, while lead (<LOD-0.58 mg/L; RQ=2,065) and cadmium (<LOD-0.23 mg/L; RQ=4,966) and mercury (<LOD-0.042 mg/L; RQ=11,160) dominated metal toxicity. Among CECs, trimethoprim (<LOD-1,042 ng/L; RQ=3.9), diclofenac (<LOD-174 ng/L; RQ=16.2), estrone (10-2,000 ng/L; RQ=548), and PFOS (5.66 ng/L; RQ=20.6) were major ecological risk drivers. Antibiotic-resistant genes were numerous and spiked at downstream sites. RQs exhibited limited seasonal trends but increased along an upstream-to-downstream gradient, with an astonishing cumulative ΣRQ of 18,434 at a downstream site used for informal electronic waste recycling. Compared to temperate rivers, the ecological risk in this urban tropical river was driven by nutrients and heavy metals, primarily due to limited wastewater infrastructure and informal recycling practices, respectively. Although CEC contaminant classes mirrored those found in temperate rivers, concentrations and RQs were markedly greater. Sources of contaminants (untreated municipal wastewater, unregulated artisanal industries) also differed from those typically causative in temperate rivers. This study presents the first integrated ERA for a tropical urban river, providing a scientific foundation for regulatory interventions, improved wastewater treatment, and ecological restoration in developing regions.

2.04.B.T-02 Context-dependent Invertebrate Biomarker Responses to Micropollutant Mixtures Across Aquatic Agroecosystems: Insights from Lentic Small Water Bodies

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Lentic small water bodies (LSWBs) are highly sensitive to the combined influence of chemical mixtures and multiple environmental stressors, yet their spatiotemporal dynamics remain poorly characterised. In agricultural landscapes, LSWBs may concentrate complex contaminant mixtures composed of both pesticides and their transformation products (TPs), alongside natural stressors such as variable oxygen levels. These interacting pressures may generate context-dependent biological responses that may not be predicted from exposure to single contaminants alone.

This study investigates how spatial variation in pesticide mixtures and associated stressors shapes the ecological responses of a sentinel invertebrate (i.e., *Gammarus roeseli*). Active biomonitoring was conducted across LSWBs (specifically fishponds) differing in land-use influence, where caged freshwater amphipods were exposed for several days. Water quality was characterised through targeted chemical analysis of pesticides and their TPs, while biological responses were assessed using a multibiomarker approach integrating antioxidant defence, neurophysiology and energy metabolism.

Clear spatial contrasts emerged among ponds, both in chemical mixtures and in biological responses. TPs represented a substantial fraction of total pesticide occurrence, including compounds derived from herbicides intensively used in surrounding watersheds. The biomarker patterns observed enabled the discrimination of sites with elevated concentrations of these TPs and highlighted oxidative stress and metabolic disturbance in exposed organisms. In LSWBs subject to additional environmental pressures such as reduced dissolved oxygen levels, biological alterations were amplified despite comparable contaminant loads, revealing environmentally driven and potentially non-additive effects.

Our results show that integrating active biomonitoring with contaminant profiling provides a refined understanding of how spatially structured mixtures and co-occurring stressors interact to affect aquatic organisms. This approach improves ecological relevance in risk assessment by capturing real-world exposure scenarios and supports the identification of vulnerable understudied freshwater habitats within agricultural landscapes.

Disclaimer/Disclosure:

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2.04.B.T-03 The Ecosystem Function of Microbial Leaf Decomposition in Streams Responds Stronger to Land Use than to Pesticides

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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. Agricultural streams are subject to multiple stressors, including pesticide exposure, excessive nutrients and habitat degradation. Those stressors can positively (e.g., moderate nutrient exposure) or negatively (e.g., pesticide toxicity) impact organisms such as aquatic fungi which then has consequences on matter decomposition. To date, most field studies investigating multiple stressor impacts have exclusively focused on the ecosystem function of leaf decomposition, without considering associated proxies for the capacity of fungal communities to decompose leaves, such as the fungal biomass or the activity of exo-enzymes. In a German-wide small stream monitoring (KgM) with most stream sampling sites located adjacent to agricultural land use, we aimed to identify the environmental variables that exert the strongest association with microbial leaf decomposition, fungal biomass and exo-enzyme activities. We quantified water quality parameters spanning from a range of chemicals including fungicides, nutrients, trace elements, to several basic physicochemical properties and determined the ratio of agricultural land use in the catchment was determined per site. Between May and July of 2018 and 2019, we deployed fine mesh bags filled with black alder leaves to determine the microbial leaf decomposition, fungal biomass (as ergosterol concentration) as well as exo-enzyme

activity. In contrast to expectations and previous findings, neither the microbial decomposition nor the fungal biomass was related to the potential fungicide toxicity towards fungi, but to the agricultural land use. The land use can be considered a proxy variable integrating various anthropogenic environmental changes. The exo-enzyme activity was associated with variables, such as water temperature, oxygen, conductivity or pH, which directly influence the production and stability of enzymes. This study highlights that the ecosystem function of decomposition in streams is influenced by agricultural land use, but not as expected by potential fungicide toxicity. Additional endpoints provided further insight into the mechanisms of decomposition and showed the relevance of single variables in the process of decomposition. The fact that decomposition was maintained despite multiple stressor effects hints towards a substantial functional redundancy of fungal communities.

2.04.B.T-04 Beyond Additivity: Interactions Between Environmental Stressors and Pesticides

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Global biodiversity is declining at an unprecedented rate in response to multiple 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* as a model and quantified responses to a single pesticide (esfenvalerate) and a field-realistic mixture of 13 pesticides under two climate-related stressors: food limitation and elevated temperature. The pesticide mixture followed Concentration Addition (CA), whereas the environmental stressors produced strong synergistic effects that were accurately predicted by the Stress Addition Model (SAM). Food limitation increased toxicity four-fold for esfenvalerate and ten-fold for the mixture (MDR = 4 and 10). Elevated temperature (+5 °C) further intensified these interactions, yielding MDRs of 19 and 42, with latent synergism strengthening through the 21-day test. Notably, the 19-fold synergism resulted almost entirely from the two non-chemical stressors; adding pesticide mixtures merely doubled the effect. Consequently, LC50 values declined sharply – from TU 3.46 to 0.18 for esfenvalerate and from TU 2.85 to 0.068 for the mixture – while LC10 values dropped by more than two orders of magnitude. Food limitation and warming were the dominant drivers of synergism, whereas pesticide mixtures contributed comparatively little. Our findings demonstrate that environmental stressors can drastically magnify pesticide toxicity. Thus, ecological risk assessments must explicitly incorporate both chemical mixtures and co-occurring environmental stressors to avoid underestimating ecological impacts.

2.04.B.T-05 Linking aquatic and terrestrial decomposition responses to hydrological extremes and pesticide exposure

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Rivers are characterised not only by longitudinal flow but also by vertical and lateral dimensions. The lateral dimension links aquatic and terrestrial ecosystems through reciprocal subsidies. Leaf litter decomposition plays a key role in carbon and nutrient cycling, yet it is often studied in isolation in aquatic and terrestrial systems, with limited research spanning the aquatic–terrestrial continuum. Both ecosystems are increasingly exposed to anthropogenic stressors and climate change, including chemical pollution and extreme hydrological events. We conducted a mesocosm experiment to investigate the effects of contrasting hydrological events (floods and droughts) and pesticide contamination originating from leaf litter collected in agricultural riparian areas on decomposition and associated communities (invertebrates and microorganisms) along the aquatic–terrestrial gradient. To reflect environmentally relevant conditions, leaf litter was collected from both forested and agricultural sites, the latter containing pesticide residues acquired in situ. The experiment lasted 25 days and comprised four phases: acclimation (10 days), drying (5 days), flooding (5 days) and recovery (5 days). Litter was exposed to drying and flooding, individually and in combination, at three distances perpendicular to flow: within the stream (0 m), on the shoreline (0.5 m) and in the riparian zone (5 m). We are quantifying decomposition rates and characterising microbial (fungal and bacterial) and invertebrate communities to evaluate how hydrological extremes and pesticide-laden litter interact to influence ecosystem processes. Pesticide content will also be analysed at the beginning and end of the experiment to assess potential changes over time. Outcomes are expected to provide new insights into how climate- and human-induced stressors alter cross-boundary ecosystem functioning, addressing key knowledge gaps related to global change in interface habitats.

2.04.P Ecological Effects of Chemical Mixtures and Multiple Stressors with Special Emphasis on Space and Time

2.04.P-Mo107 Aquatic invertebrates under pressure: The interplay of temperature and receptor binding in bioaccumulation

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Chemical pollution and climate change are major planetary boundaries exceeding the safe operating space the. Yet, the interaction between contaminant exposure and climate conditions remains poorly understood, as laboratory predictions often underestimate environmental risks. This study examines temperature-dependent toxicokinetics of the neonicotinoid thiacloprid in the aquatic invertebrate *Gammarus pulex* to improve predictions of bioaccumulation across laboratory and field conditions. Neonicotinoids show strong binding to nicotinic acetylcholine receptors (nAChRs), which may reduce elimination and enhance accumulation at higher temperatures.

Toxicokinetic experiments were conducted under constant temperatures (6–21°C) at 50 µg/L (one day uptake, three days elimination). Additional receptor binding assays quantified membrane-bound fractions. Chemical analyses were performed using online-SPE coupled with LC-HRMS/MS. A multicompartment toxicokinetic model was applied and combined with the Arrhenius equation to describe temperature-dependent rate constants. Model validation took place in stream mesocosms with thiacloprid spikes (10 µg/L) under ambient and heatwave (+8°C) conditions.

The two-compartment model fitted laboratory data well ($R^2 = 0.80\text{--}0.98$) accurately predicted thiacloprid concentrations. Temperature dependencies of toxicokinetic rate constants (Arrhenius temperature $TA = 7900 \pm 500 \text{ K}^{?1}$) matched physiological expectations and observations from previous experiments. Most residual thiacloprid remained bound to membrane proteins, explaining its persistence. In mesocosms, tissue concentrations were almost twice as high under heatwave conditions ($46 \pm 10 \text{ µg/kg}$) compared to ambient conditions ($25 \pm 6 \text{ µg/kg}$). After exposure, the concentration in the gammarids remained constant with no observed elimination. This indicates a higher body burden – lasting far longer than the event of exposure – if neonicotinoid exposure and heatwaves cooccur simultaneously, such as in typical pesticide application seasons such as late spring and summer.

Our results advance the mechanistic understanding of chemical exposure and fate under changing climatic conditions and highlight how heat events can intensify risks caused by neonicotinoids. These findings emphasize the need for regulatory strategies addressing long-term ecological impacts in a warming climate.

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2.04.P-Mo108 Bugs on drugs: Short-term Exposure of Fluoxetine and Clobazam impacts the Activity and Foraging Behaviour of the Northern Damselfly under different Temperature Scenarios

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Freshwater systems are increasingly contaminated with pharmaceuticals and personal care products and are simultaneously affected by rising global temperature, placing roughly a quarter of their fauna at risk of extinction. Psychoactive pharmaceuticals are of particular concern because they are biologically active at low concentrations, persist in the environment, and often co-occur as mixtures. But despite their widespread occurrence, we still lack a clear understanding how warming alters the behavioural effects of these pharmaceuticals—especially when organisms are exposed to multiple compounds simultaneously.

Therefore, we conducted a controlled laboratory experiment using northern damselfly nymphs—a widespread aquatic predator sensitive to environmental change—to test how a two-week exposure to two psychoactive pharmaceuticals (Fluoxetine and Clobazam), individually and in mixture, affect their activity and foraging behaviour under two temperature scenarios. Fluoxetine reduced attack success at low temperature, while Clobazam consistently decreased movement speed across temperatures. The mixture did not produce clear interactive effects.

Overall, our findings show that temperature modulates the behavioural impact of pharmaceuticals, with potential

implications for predator-prey interactions and ecosystems functioning. This underscores the need to consider interactive stressors—especially warming-pollutant interactions—in future ecological risk assessments.

2.04.P-Mo109 Multistress in *Chironomus riparius*: Response of Endocrine-Related Genes to Chemical Mixtures and Temperatures

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Pollution and climate change are among the main concerns today. There is a lack of knowledge about the mechanisms underlying responses to chemical mixtures when temperatures change, a condition that will occur under current climate change. The gap is especially evident in aquatic invertebrates, which are essential in the food web of freshwater ecosystems. To gain insight into how invertebrates can manage pollution during climate change, the effects of single, double, and triple mixtures of bisphenol A, cadmium, and endosulfan at four temperatures (18, 21.5, 23, and 25 °C) have been studied. Five receptor genes related to the endocrine system (EcR, Met, InR, MAPR, and AllaR) were selected to examine the impact of the three selected chemicals on their endocrine-disrupting activity. Fourth instar larvae were exposed for 1, 2, and 4 days and frozen individually. The RNA was extracted and retrotranscribed to analyze the mRNA levels of the five genes selected by Real-Time PCR. As reference genes, actin and glyceraldehyde-3-phosphate dehydrogenase were used. The results show the differential impact of time, temperature, and chemical stressors, opening new perspectives for mimicking the wild conditions organisms encounter. In addition, the complexity of result interpretation is highlighted by the number of variables implicated and the potential events that can occur in the cell during short-, medium-, and long-term molecular responses.

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2.04.P-Mo110 Multi-generational Sub-lethal Effects of Fungicide Tebuconazole Under Climate Change Scenarios in *Daphnia magna*

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European freshwater streams are regularly exposed to multiple stressors including a multitude of chemicals designed for agricultural use. These chemicals, such as pesticides, are known to inhibit and cause a range of defects in the reproduction of freshwater organisms. Chronic pesticide exposure, which occurs due to the repeated application to crops across several seasons, in particular is of high interest due to the potential interaction with other stressors. Thus, seasonal and daily changes in variables such as temperature might cause altered effects of pesticides on freshwater organisms. Thermal fluctuations can have an effect on pesticides availability for uptake, its distribution and metabolism, as well as general organismal functions of different invertebrates. We assessed how changes in temperature can influence effects of pesticides on a macroinvertebrate. We performed a multi-generational study on *Daphnia magna* using the fungicide tebuconazole. Tebuconazoles is widely used as a systematic fungicide and was shown to cause oxidative stress generation and neurotoxic effects in macroinvertebrates. Therefore, it is likely to impact multiple trophic levels within an ecosystem. We extended the pre-established OECD 211- protocol to assess three generations of *Daphnia magna*, allowing us to study chronic propagation effects. For all generations, we collected data on multiple sub-lethal parameters, such as size, swimming behavior and oxidative stress markers in addition to mortality, produced sex ratios and juvenile production. To assess the possible temperature-dependent effect modulation, three different temperature conditions were evaluated with decreased (16°C), laboratory culture standard (20°C) and increased (24°C) temperatures representing a realistic fluctuation within a waterbody We hypothesize that both 16°C and 24°C-temperature conditions will lead to decreasing effect concentrations. During the conference, we present and discuss our findings in light of risk assessment in the context of multiple stressors.

2.04.P-Mo111 Seasonal Context Modulates Multiple-Stressor Effects on Aquatic Microbial Function

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Microbially-driven leaf litter decomposition (LLD) is an important ecological function in streams that can be altered by multiple stressors originating from agricultural runoff, such as pesticides, nutrients and suspended solids. However, seasonal shifts in leaf-associated microbial communities, which is triggered by the seasonal context in terms of temperature, flow, and nutrient availability, may change the community's stress-tolerance. We expected fungicides and suspended solids to reduce and nutrients to enhance LLD, with effect sizes being dependent on the season during which the experiment is performed. Therefore, we conducted a 4×3×3 factorial experiment over 21 days over four seasons in 2024. The three factors included a fungicide mixture (4 levels), nutrients (3 levels), and suspended solids (3 levels), while microbial communities were sampled during winter, spring, summer and autumn from the same near-natural site. LLD was selected as the functional variable, while structural changes will be assessed using molecular techniques. We observed that higher levels of fungicides and suspended solids reduced LLD, while nutrients availability stimulated it, supporting our hypothesis, although the effects varied seasonally. The highest fungicide treatment (600 µg/L) reduced LLD in winter (?7%), summer (?2%), and autumn (?17%), but increased it in spring (+7%) compared to controls. At the same time, the high solids treatment (100 mg/L) reduced LLD in winter and spring (?7%), while no significant effect was observed in summer or autumn. Elevated nutrient concentrations (NO₃-N/PO₄-P: 18/1.8 mg/L) increased LLD significantly across all seasons. Seasonal differences in LLD in response to the applied stressors likely reflect temporal changes in microbial community structure and thus their successional dynamics, which influence their functional sensitivity over time. We will verify this by analysing the microbial community structure across seasons using quantitative real-time polymerase chain reaction (qPCR).

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2.04.P-Mo112 Distance-decay Patterns of Riverine Microbial Communities and Their Biodegradation Potential

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Microorganisms are fundamental to the biodegradation of micropollutants, a process that underlies the self-purification capacity of river systems. Understanding their dynamics and the factors influencing their functionality is critical, as alterations in microbial diversity and composition can directly affect purification efficiency. This study investigated changes in benthic and pelagic microbial communities along a 5?km stretch of the wastewater-impacted Örsunda River, focusing on spatial distance-decay patterns before and after a flood event. We hypothesized that distance-decay would occur in both environments and that flooding would attenuate these patterns. Microbial communities were profiled using 16S rRNA metabarcoding, while biodegradation potential was evaluated with the modified OECD 309 test. Analyses revealed distinct differences in diversity and composition between benthic and pelagic communities, both of which exhibited distance-decay relationships. Notably, flooding weakened these spatial patterns. Across the 5?km transect, communities degraded 57 compounds, of which 11 showed apparent spatial variation. Preliminary evidence further indicated that flooding altered the biodegradation of specific compounds. These findings highlight the need to consider spatial distance and disturbance regimes when assessing the ecological impacts of Wastewater Treatment Plants. They also suggest that biodegradation studies should ideally be conducted during periods of minimal disturbance to ensure ecologically meaningful interpretations.

2.04.P-Mo113 SPATIOTEMPORAL FACTORS RELEVANT IN THE POPULATION DYNAMICS OF SNAIL HOST IN RELATIONSHIP TO SCHISTOSOMIASIS TRANSMISSION

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Schistosomiasis remains a public health problem in Kenya where ~4.3 million people require preventative chemotherapy. Recent research highlighted the role pesticide pollution can play in the transmission of the disease, by disrupting the ecosystem and favoring the host snails over their competitors. Here, we expand on that research by carrying out surveys along 5 rivers over the course of a year (2 wet and 2 dry seasons),

measuring snail incidence & abundance in comparison to predator & competitor dominance and pesticide pollution. We observed that host snail abundance increases with pesticide pollution ($R = 0.23$, $P = 0.009$) but decreases with competitor dominance ($R = 0.15$, $P = 0.006$) in the wet seasons. We also observed that both predator ($R = 0.34$, $P < 0.001$) and competitor dominance decreased with T_{Umax} in the same period ($R = 0.27$, $P = 0.004$). From these, we postulate that pesticide pollution contributes to increased *Schistosoma*-host snail abundance, primarily by reducing the dominance of their competitors. The study adds evidence to the hypothesis that pesticide pollution leads to more favorable conditions for the host snails, including for the first time, temporal evidence showing seasonal influence, increasing disease risk in areas where agriculture and the host snail habitat intersect.

2.04.P-Mo114 Balancing Risk and Vermiremediation: Temperature Effects on Heavy Metal Removal by *Eisenia fetida* in Sewage Sludge-Amended Soils

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The increasing generation of sewage sludge (SS) poses growing challenges for its safe and sustainable management. While SS can be reused in agriculture for its nutrient-rich organic matter, these benefits are counterbalanced by contaminants such as heavy metals (HM) and other potentially harmful substances. Current regulations often fail to capture broader ecological risks to soil organisms and ecosystems. Vermiremediation, the use of earthworms, sustainably removes pollutants and enhances soil properties (e.g., pH, organic matter content). Climate change may alter HM mobility, availability, toxicity, affecting soil organisms and bioremediation potential. It is therefore crucial to understand how temperature influences earthworm activity, survival, and overall vermiremediation efficiency.

In this study, we examined the effect of temperature on vermiremediation in soils amended with SS from a municipal wastewater treatment plant serving 10,000–20,000 population equivalents (PE), containing Co, Mn, Cr, and Cu. Two temperature regimes (21 °C and 25 °C) were tested under optimal soil water content (45–50% of maximum WHC) using compost earthworms (*Eisenia fetida* L.). HM accumulation, survival, and weight gain were monitored to assess how temperature affects remediation performance.

The results showed that *E. fetida* tolerated low HM mixtures under both temperatures. SS dose, temperature, and their interaction significantly affected survival (Factorial ANOVA, $F = 66.66$ – 86.23 , $p < 0.05$). At 21 °C, low SS doses (12.5 and 25 t/ha) promoted growth (18–25% weight gain), while the highest dose (50 t/ha) caused the lowest survival. Elevated temperature (25 °C) intensified HM toxicity, reducing both survival and growth. One-way ANOVA showed that sludge dose significantly influenced HM removal: Co and Mn were removed efficiently (>80%), while Cr (20.8–35.2%) and Cu (17.9–40.3%) showed lower removal, particularly at higher SS doses and elevated temperature.

Careful management of environmental conditions and pollutant levels is essential to maintain soil organism health and ensure effective remediation. These findings provide valuable insights into how temperature modulates vermiremediation in SS-amended soils, emphasizing the need to consider climate-related factors when applying biological remediation. They have important implications for optimizing future bioremediation strategies and ensuring the safe agricultural reuse of SS.

2.04.P-Mo115 Mixture Effects of Metal-Organic Pesticides on a Freshwater Ecosystem: A Microcosm Study

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Chemical pollutants in the environment rarely occur as single compounds. Runoff from agricultural fields often introduces pesticide mixtures into freshwater systems, yet current regulatory guidelines typically evaluate toxicity of individual active substances only. This method neglects potential mixture effects, which can influence toxicity directly, as well as cause indirect ecological interactions across the food chain. These oversights of particularly the ecological interactions may lead to inaccurately predicted toxicity with the standard reference models Concentration Addition (CA) and Independent Action (IA).

To address these gaps, a three-month microcosm experiment was run to investigate the combined effects of an organic fungicide, azoxystrobin, and the metal, copper, on freshwater ecosystems. The study looked at both direct and indirect effects on algal population density, macrophyte growth, macroinvertebrate populations, and zooplankton populations and communities. Comparing observed outcomes with expectations from single

chemical laboratory assays, we assessed whether standard regulatory methods can capture mixture effects on an ecosystem level.

The results showed that when chemicals were applied in the microcosms at concentrations proven to have effects in the lab, no significant effects were detected. Instead, organism effects were more strongly influenced by environmental parameters such as heatwaves and nutrient limitations. This indicates that once dynamic ecological variables are introduced, current toxicity assays fail to predict ecosystem-level outcomes.

Furthermore, it shows the urge to take environmental stressors into account, besides chemical stress alone.

However, as the laboratory results showed the highest toxicity, and strongest mixture effects, using lab-data to set ecological limit values would still be a protective strategy.

Disclaimer/Disclosure:

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2.04.P-Mo116 Developing a Monitoring Framework for UK Protected Waters: Integrating Chemical Surveillance and eDNA to Identify Emerging Risks to Sensitive Species

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Freshwater ecosystems in the UK host a high proportion of nationally important and vulnerable habitats, yet the pressures from agricultural, veterinary, and wider land-use chemicals remain poorly characterised at catchment scale. Regulatory monitoring typically focuses on a suite of priority substances to inform on water quality but tends to lack parallel ecological parameters, leaving major uncertainties regarding the occurrence, mixture exposures, and ecological relevance of many pesticides and veterinary medicines with plausible pathways to protected sites. Furthermore, it is rare that monitoring programmes are designed to ensure a robust integration of both chemical surveillance and biomonitoring to identify the impact of chemical pressure to vulnerable species and communities. To address these gaps, we are developing a monitoring programme that integrates targeted chemical surveillance with biomonitoring metrics derived from environmental DNA (eDNA) to provide reliable and robust data for environmental risk assessment.

We first undertook a systematic process to identify and prioritise chemicals of interest, combining usage indicators, physicochemical properties (persistence, mobility, bioaccumulation potential), and ecotoxicological endpoints relevant to the specific aquatic habitats (e.g. the Pesticide Load Index). Both passive and spot-sampling methodologies were evaluated to determine feasible approaches for capturing low-level, intermittent, and mixture exposures in flowing waters. A parallel examination of traditional and an eDNA-based biomonitoring strategies was also conducted and brought together with the chemical monitoring approaches to design a monitoring programme designed for the assessment of community-level responses and the detection of sensitive or declining taxa potentially at risk from chemical pressures.

Here we present the development of a forthcoming UK monitoring project aiming to (i) establish practical sampling designs that reflect realistic chemical exposure profiles; (ii) develop approaches for linking chemical occurrence with biodiversity indicators to strengthen causal inference; and (iii) design a robust monitoring programme that will provide a transparent evidence base for spatiotemporal effects of chemical mixtures on aquatic communities and provide data for environmental risk assessment which can then in turn inform future UK monitoring and regulatory protection strategies for freshwater habitats.

Disclaimer/Disclosure:

UK PLI database used for prioritisation was produced and provided by Defra, Fera, and the University of Hertfordshire

2.04.P-Mo117 Exposure to Chemical Mixture Triggers Oxidative Stress Biomarkers under Environmental Stress

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Natural ecosystems include multifactorial stressor mixtures that can induce sublethal effects at concentrations far below lethal levels, yet their long-term physiological consequences remain poorly understood, thereby challenging the traditional risk assessment framework. In this study, we investigated the chronic effects of combined chemical and environmental stressors on the freshwater cladoceran *Daphnia magna*, focusing on how molecular stress biomarkers can be used as predictors for individual survival. Organisms were exposed continuously for 21 days to copper and food limitation, with an additional 24-hour pulse exposure to the pyrethroid insecticide esfenvalerate. Survival was modeled using CA, EA, and SAM approaches across different time points, with SAM providing the most accurate predictions of combined stressor effects. The addition of 0.1 µg/L esfenvalerate to the combination of copper and food stress accelerated the onset and increase of synergistic effects (MDR EA = 2.46) compared to copper and food stress alone (MDR EA = 1.61). At the molecular level, activities of the oxidative stress biomarkers catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST) were quantified. Significant upregulation of CAT and GST activities was observed only under multiple-stressor exposure (two-way ANOVA, $p < 0.01$ and $p < 0.001$). Moreover, enzyme activity exhibited a significant negative correlation with survival in copper + food stress and copper + food stress + esfenvalerate treatments ($R^2 > 0.2$, $p < 0.001$), suggesting biomarker responses serve as early indicators of physiological stress preceding mortality only when food stress is present. These findings reveal how environmental stressors modulate oxidative defense systems under chemical stress, highlighting the importance of food restriction to reveal the response of stress biomarkers. Overall, the results enhance understanding of toxicity and defense mechanisms under continuous and pulse exposures of differently acting chemicals.

2.04.P-Mo118 Linking life-history shifts and gut microbiome restructuring in *Daphnia magna* under combined nanoplastic–antibiotic stress

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Nanoplastics (NPs) can modify the toxicity of co-occurring pollutants, including antibiotics, yet their combined impacts on freshwater keystone grazers and their associated microbiota remain poorly understood. We tested how spherical polystyrene NPs and the fluoroquinolone enrofloxacin jointly affect life-history traits of the planktonic crustacean *Daphnia magna* and the metabolic and taxonomic structure of its gut microbiota, aiming to link organismal performance to microbiome-level mechanisms relevant for eco-evolutionary responses. In a factorial experiment with multiple NP and ENR concentrations, we quantified (i) body size and reproductive traits (clutch size, egg volume, and total reproductive investment), (ii) gut microbial metabolic diversity (respiration rate and relative use of different carbon sources), and (iii) gut microbial taxonomic diversity. Both stressors alone significantly affected most life-history and microbiome parameters, and their effects were consistently interactive across all measured endpoints. Nanoplastics and enrofloxacin reduced *Daphnia* body size and reproductive investment, indicating fitness costs of exposure, while simultaneously reshaping gut microbiome metabolism and composition. NP exposure generally increased the metabolic rate of the gut microbiota whereas enrofloxacin decreased it, and co-exposure altered the relative use of carbon substrates and increased the contribution of Firmicutes in the gut community. These patterns support a “Trojan horse” and interaction-based view of nanoplastic–antibiotic effects, in which contaminant mixtures generate non-additive impacts on host fitness and microbiome structure. Overall, our study highlights mechanistic links between individual life-history responses and microbiome restructuring under combined chemical stress, providing a framework for integrating host–microbiome dynamics into predictive, evolution-aware ecotoxicology.

2.04.P-Mo119 From acute stress to community-level adaptation: eco-evolutionary responses of lake microbiota to nanoplastic–enrofloxacin co-exposure

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Nanoplastics (NPs) and antibiotics co-occur in freshwaters, yet their time-resolved, joint impacts on microbial communities, and the scope for rapid community-level adaptation, remain poorly constrained. We exposed lake microbiota to polystyrene NPs (~420 nm; 10^3 – 10^7 particles L⁻¹) and enrofloxacin (ENR; 10 or 100 ng L⁻¹) for 14 days, tracking abundance, per-cell respiration, community-level physiological profiles (CLPP), and taxonomy. By day 7, both stressors reduced abundance, with stronger ENR effects; CLPP was depressed mainly by ENR, whereas per-cell respiration increased in several NP×ENR combinations, and substrate use shifted transiently from complex carbons toward simpler substrates (notably amines), consistent with an acute stress response. By day 14, population-level responses attenuated, but community structure diverged: α -diversity remained depressed only under high ENR with NPs, β -diversity resolved into three distinct assemblages (enriched Sporichthyaceae and Comamonadaceae; reduced Burkholderiaceae and Verrucomicrobiaceae), and Actinobacteriota remained dominant. A Mantel test indicated that taxonomic restructuring increasingly explained CLPP at day 14, pointing to community reassembly and selection for stress-tolerant lineages that partly restore function under continued exposure.

A complementary fate experiment showed that dissolved ENR declined strongly over days, more rapidly in bacteria-present (unfiltered) water and further in the presence of NPs, with several NP-amended, unfiltered treatments approaching the analytical LOD by days 7–14. A 4 h assay detected no treatment differences, indicating negligible immediate photolysis or rapid sorption and implicating biological sinks that strengthen over multi-day, multi-generation timescales. Together, these results show that NP–antibiotic interactions are time-dependent and sometimes antagonistic at the population level, yet drive lasting community reassembly and altered contaminant fate. Our time-resolved, multi-level assessment illustrates how eco-evolutionary dynamics of freshwater microbiomes can decouple early physiological stress from later partial functional recovery, with implications for predicting long-term ecological risk of contaminant mixtures under global change.

2.04.P-Mo120 Modelling Chemical Mixtures and Disentangling its Effects on River Ecology in a Multiple Stressors Context

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River ecosystems are shaped by a multitude of processes that operate across a hierarchy of spatial scales, from local to regional factors. In this context, identifying how chemical pollution shapes community composition is challenging, notably due to the diversity of chemicals released into the systems, the scarcity of large-scale monitoring programs that jointly assess chemical pollution and biota, and the interactions between pollutants and other environmental drivers.

In this study, we examined relationships between catchment land-use and water chemistry, which were subsequently used to evaluate large scale links between chemical and biological patterns in European rivers. In detail, we used *in silico* ecotoxicological predictions to evaluate potential toxicity of chemical exposure from European monitoring databases such as the NORMAN EMPODAT database and WISER. We established toxicity estimates independently for different chemical classes of hazardous substances such as pharmaceuticals, metals or biocides. The potential toxicity of each chemical class and the joint toxicity was then linked to detailed land-use descriptors to predict the toxicity at unmeasured locations. In the next step, we examined associations between the estimated potential toxicity and ecological communities. We considered the four biological quality elements defined by the Water Framework Directive, namely fish, macroinvertebrates, diatoms and macrophytes. Finally, we modelled functional and taxonomical community composition as a function of land-use, different variants of estimated potential toxicity, environmental parameters (e.g. nutrients, general physico-chemistry, discharge), and ecological processes such as competition. Thus, we integrated chemical, environmental, and biotic drivers into a single analytical framework. We tested the hypothesis that the explanatory power of estimated potential toxicity for community composition is negatively associated with the explanatory power of local environmental and ecological processes. In other words, land-use drive toxicity is hypothesised to mask local processes shaping communities. We discuss how land-use can be used to predict chemical mixture patterns and, ultimately, improve our understanding of the relevance of chemical emissions in a multiple stressors context.

2.04.P-Mo121 Chemical Mixtures in European River Basins: A Probabilistic Risk Model for Integrating Additive and Independent Effects

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Chemical mixtures and potential cocktail effects in aquatic ecosystems are recognised as a threat for river basins across Europe. Risk assessment for chemical mixtures has been subject to much development and debate in the last decade, but many challenges remain. Probabilistic risk methodologies are becoming more common in both human and environmental risk assessment (ERA) and offer new opportunities for fundamental challenges in ERA, such as the characterisation of mixture risk. The Concentration Addition (CA) concept is commonly used in lower-tier 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).

We have developed a hierarchical probabilistic model for integrating these two concepts, formulated as an object-oriented Bayesian network (OOBN). First, probabilistic risk quotients (RQ) are calculated for individual chemicals, as probability distributions of environmental concentrations divided by a threshold environmental value. Next, the CA concept is applied within groups of substances (e.g., with similar mode of action) by summing the RQ distributions. Finally, the IA concept is applied across the different substance groups, to combine RQ distributions by joint probability calculation ("OR" expressions). This approach to mixture risk calculation was first developed for nature-based wastewater treatment systems in the EU project MULTISOURCE. In the ongoing project ENCORE (ENVIRONMENTAL CO-exposure and Risk Estimation) we will apply the same approach at larger spatial and temporal scales. We obtain predicted exposure concentrations data from the ENCORE fate model, a process-based model for simulation of environmental concentrations in river basins across Europe (to be presented in a different session).

Here we present a pilot study for a subset of the substances (15 pesticides) and river basins (in Belgium), as a proof-of-concept. The aims of this presentation are: (1) to demonstrate a novel probabilistic approach to mixture risk characterisation, which integrates the CA and IA concepts in a hierarchical probabilistic model; (2) to demonstrate the applicability of the model to the pilot study; and (3) to consider the potential for upscaling this approach to larger spatial and temporal scales.

Disclaimer/Disclosure:

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2.05 Extreme Events in Remote Ecosystems: Environmental Chemistry and Ecotoxicological Responses

2.05.T-01 Effects of Hydrological Extreme Events on Organic Micropollutants in a Large River

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Heavy rain events, floods and droughts will likely increase in the future due to climate change. These events can have a profound impact on freshwater quality including concentrations of organic micropollutants (OMPs). The data basis on event-based pollution with OMPs is particularly sparse for large rivers. Here, high time-resolved sampling and comprehensive screening approaches, such as suspect and nontarget screening (NTS), are required. In this study, we investigated pollution patterns of OMPs in the Moselle River during rain events, a flood and a low flow event. Daily composite water samples were collected at a monitoring station in Koblenz (Germany) in 2021 and 2022 and subjected to NTS and target analysis. During a heavy rain and flood event in July 2021, three pollution patterns were identified in NTS data reflecting i) wastewater and immediate surface runoff emissions, ii) groundwater emissions and iii) flood-related emissions from agricultural and forested areas. The event led to high concentrations of pesticides and their transformation products (TPs) and affected water quality for several weeks. In summer 2022, a 67-day period of low flow and an intensive cyanobacteria bloom occurred. Again, OMPs were sorted into three groups. i) Persistent wastewater-related OMPs increased and ii) rather degradable OMPs decreased during low flow. Opposing trends of some pharmaceuticals and their TPs were observed. This suggests enhanced transformation processes in wastewater treatment plants or in the environment. The third pattern summarised OMPs which decreased during the monitoring period, e.g., pesticides emitted via surface runoff and groundwater. The generally opposing behaviour of wastewater- and groundwater-related pollutants was observed in the drought and flood event and was transferable to smaller rain events and long-term monitoring data. Our studies showed that hydrological and meteorological (extreme) events can considerably affect the pollution with OMPs in large rivers – calling for adjustments in future monitoring, e.g., by implementing continuous and flow-proportional sampling and using pattern-representative OMPs and TPs as indicators for pathway-specific contributions and altered transformation processes. To gain a

deeper understanding on the impact of droughts and algae blooms, unannotated, drought-related features are currently investigated. Further, event-based exposure scenarios should be considered in ecotoxicological studies.

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2.05.T-02 Sublethal Chemical Exposure Decreases Thermal Tolerance and Increases Variability Differentially in Freshwater Fish and Invertebrates

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In environments increasingly afflicted by extreme climatic events, a considerable fraction of organisms are likely to experience temperatures approaching their physiological limits while simultaneously exposed to chemical pollution. Realistic contaminant exposure conditions for freshwater organisms often entail exposure well below lethal thresholds, yet such sublethal exposures may still prove harmful, especially in conjunction with extreme temperatures. The impacts of chemical contaminants on organism's upper thermal tolerance are understudied relative to the effects of temperature on pollutant toxicity. These have not yet been quantitatively analysed across different species, despite increasingly frequent extreme heat events. To fill this gap, we set out to determine whether sublethal chemical exposure changes the upper thermal tolerance of freshwater invertebrates and fish using a meta-analytic approach. We test the hypotheses that sublethal chemical exposure will change upper thermal tolerance and increase variance thereof, and that the magnitude and direction of these changes depends on stressor intensity and taxonomic group (fish or invertebrate) with a three-level random effects meta-analysis and meta-regression. Sublethal chemical exposure decreased upper thermal tolerance overall for fish and invertebrates, with an overall effect estimate of $-1.14\text{ }^{\circ}\text{C}$ (-1.66 to -0.62 95% CI, $p < 0.0001$). The meta-regression with log toxic units (logTU) displayed a significant negative relationship, with greater reductions in upper thermal tolerance with increasing logTU (estimate -0.85 , $p = 0.0008$). The effect of sublethal chemical exposure on the log coefficient of variation ratio differed significantly between fish (0.91) and invertebrates (0.46), indicating a greater increase in variability of upper thermal tolerance with sublethal chemical exposure in fish than in invertebrates. Therefore chemical pollution potentially impedes freshwater organisms' ability to cope with extreme temperature events, and the increased variability found in fish could complicate predictions of sublethal chemical effects. However, mechanisms governing these effects have not yet been elucidated, and studies conducted with a variety of additional species and chemicals are needed to confirm this pattern.

2.05.T-03 Spatial Patterns and Dynamics of Organic Contaminants in Urbanized Rivers Under Drought Conditions

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One of the most pressing concerns for urban river systems is the pollution with organic contaminants. In addition, river water quality is affected by extreme weather events. Drought conditions are known to exacerbate the deterioration of water quality in terms of nutrient concentrations, but the effects of droughts on the concentration of organic contaminants such as pharmaceuticals and industrial chemicals in rivers have not yet been quantified. Conceptually, reduced river flows can decrease the dilution capacity of rivers, resulting in higher concentrations of pollutants, particularly those originating from continuous emissions through wastewater treatment plants (WWTPs). Simultaneously, low water levels and long retention times in combination with increased sunlight penetration during droughts might enhance the photochemical transformation of organic contaminants. Thus, the goal of this study was to determine and quantify how the organic contaminant concentrations and compositions in urban rivers shift during drought periods compared to conditions of mean river flow. To this end, we conducted five sampling campaigns along the Neckar River during drought periods and three under mean discharge conditions between May and November 2025. The sampling sites were located upstream as well as 500 and 2000 m downstream of municipal WWTPs where the ratio of effluent to river discharge is high. All samples were analyzed for 21 selected organic contaminants using liquid chromatography mass spectrometry. The set of organic contaminants covered compounds persistent to (a)biotic degradation, photolabile compounds as well as photo transformation products. Our results demonstrate

that concentrations of pharmaceuticals downstream of WWTPs were elevated during drought periods due to lower dilution in the river. In addition, photo transformation was enhanced during droughts, which lead to a faster decrease in contaminant concentrations down the waterline compared to mean flow conditions. Our data will flow into a model to elucidate the impact of droughts on the water quality of urban river systems under different climatic scenarios, which is important for urban water management.

2.05.T-04 Presence and Temporal Trends of Polycyclic Aromatic Hydrocarbons in Arctic Char from Remote High Arctic Lakes

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Polycyclic aromatic hydrocarbons are toxic and persistent organic contaminants that can bioaccumulate in organisms and the environment and biomagnify at the highest levels of food chain, posing ecological and human health concerns. Despite the perceived isolation of High Arctic ecosystems, recent environmental change driven by regional warming and permafrost degradation may mobilize contaminants stored in soils and sediments, increasing their availability to aquatic food webs. However, long-term trends of these compounds in Arctic biota remain poorly understood.

This study investigates temporal changes in concentrations and profiles of polycyclic aromatic hydrocarbons in Arctic char (*Salvelinus alpinus*), the only fish species present in isolated lakes of the Canadian High Arctic. We analyzed muscle samples (n=73) collected between 2008 and 2023 from two contrasting lakes on Melville Island, representing systems with different degrees of geomorphological disturbance. Concentrations were determined using gas chromatography coupled to tandem mass spectrometry, following validated extraction and clean-up procedures, and normalized to lipid content.

Results reveal clear differences between lakes. Total polycyclic aromatic hydrocarbons ranged from 12 to 900 ng g⁻¹ lipid weight, with a predominance of low and medium molecular weight compounds such as naphthalene, phenanthrene, fluorene and pyrene. The western lake showed a marked increasing trend over time, coinciding with documented active layer detachments, higher turbidity and enhanced delivery of terrestrial organic matter. In contrast, the eastern lake exhibited a progressive decrease in concentrations, suggesting a more stable system with limited remobilization from surrounding catchments.

These findings indicate that physical disturbances linked to permafrost thaw can remobilize historically deposited contaminants, increasing their bioavailability and incorporation into aquatic food webs. Understanding these processes is essential for predicting future contaminant exposure in Arctic ecosystems under continued warming.

This work advances knowledge on contaminant dynamics in changing polar environments and provides baseline information for assessing future ecological risks associated with permafrost degradation.

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2.05.P Extreme Events in Remote Ecosystems: Environmental Chemistry and Ecotoxicological Responses

2.05.P-Mo122 High-Frequency Multivariate Multi-Tracer Analysis of Combined Sewer Overflow Contributions in Small Urban Streams: Prioritising Chemical Tracers for Quantitative Source Apportionment

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Urban rivers can receive large amounts of untreated wastewater during storm events through combined sewer overflows (CSOs), causing rapid and transient spikes in chemical contaminant loads. Effects of these events are difficult to quantify with standard monitoring methods due to limiting insights into the dynamics of both pollutant concentrations and discharge volumes of CSOs and receiving rivers during storm events. The present study aims to develop a streamlined approach to track CSO contributions with high temporal resolution and identify the most effective indicators and model approaches for quantitative source apportionment in urban streams during storm events.

High-frequency monitoring was conducted at multiple locations along two small urban streams, capturing variations in water level, electrical conductivity, and turbidity. Water samples were collected at high temporal resolution both in the main channels and directly at overflow points. Samples were analysed for a selection of 32 trace organic compounds, major ions, and stable water isotopes. A multivariate mixing model was applied to jointly constrain the relative contribution of CSOs to the river water using multiple chemical tracers and discharge measurements.

Results indicate that a limited set of compounds, including commonly used artificial sweeteners and certain pharmaceuticals, provides a reliable signal to distinguish CSO water from river water. Overflow contributions were found to fluctuate substantially during storm events, with chemical tracers capturing local impacts more precisely than volumetric flow estimates.

The present study demonstrates that a focused, tracer-based monitoring strategy allows for robust and minimally invasive quantification of CSO contributions in urban streams. The approach improves understanding of short-term contaminant dynamics and offers practical guidance for managing urban water quality and prioritizing monitoring resources. Ongoing research focuses on the development of a joint multi-tracer, joint inversion approach that simultaneously integrates different chemical tracers, discharge measurements and their uncertainties.

2.05.P-Mo123 Impacts of a massive flash flood event on organic pollutant risks in a protected Mediterranean wetland (L'Albufera, Valencia, Spain)

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Flood events are becoming more frequent and severe due to climate change, with projections indicating further increases in the coming decades. On the 29th of October 2024, the Valencia region (Spain) experienced very intense rainstorms (200-700 L/m²), which resulted in a catastrophic flash flood. A significant proportion of the floodwaters and sediments generated were discharged into L'Albufera Natural Park (ANP) - a Ramsar-listed wetland. The flash flood carried a wide range of urban and industrial residues into the ANP, and produced the collapse of the sewage network, which could contribute to the release of various types of organic pollutants. This study aimed to characterize the pollutant footprint of the massive flash flood that impacted the ANP and to assess its ecological risks. To do so, a monitoring campaign was conducted after the flash flood, involving the collection of water and sediment samples from 15 locations, and the analysis of 241 organic pollutants, including pesticides (PEST), pharmaceuticals and personal care products (PPCPs), perfluoroalkyl substances (PFAS), organophosphorus flame retardants (OPFRs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs). The study shows that the combined effect of the massive flooding and the sewage system collapse led to a surge in pollutants—mainly PPCPs and PAHs—and the widespread remobilization of other hydrophobic compounds. While acute lethal effects on aquatic life were unlikely, we identified 44 OPs that surpassed the long-term aquatic toxicity thresholds. The most significant exceedances in water included ibuprofen, PFOS, and fluoranthene, while sediments contained 13 high-risk compounds (PFOS, PESTs and PPCPs). Spatially, the highest ecotoxicological risks from water exposure were in the lake and the NW sector, and from sediment were uniformly distributed. This study presents one of the first detailed investigations of extreme flood impacts on aquatic ecosystems in Europe, highlighting pollutant mixtures that are likely emitted into the environment following such extreme events. The findings offer critical insights for guiding ecosystem management in the ANP by identifying vulnerable areas and environmental compartments at risk of contamination due to flooding.

Disclaimer/Disclosure:

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2.05.P-Mo124 Local Sources vs Long-Range Transport: Cyclic Volatile Methylsiloxanes in Antarctica and the Arctic

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Cyclic volatile methylsiloxanes (cVMS) are high-production-volume chemicals used globally in personal care products and industrial applications. Their occurrence in remote environments raises questions about long-range atmospheric transport and local emissions. We present results from two studies investigating the cVMS D4, D5, and D6 in polar regions: Antarctica (2023/24) and Arctic Svalbard (2023). Comparable sampling and analytical methods were applied for air, terrestrial, and aquatic matrices to enable comparison between sites and hemispheres.

Across all Antarctic stations, D4, D5, and D6 concentrations in outdoor air were generally very low, with detectable levels primarily restricted to research-station settlements and absent or near detection limits in more remote areas. Extended winter deployments at the Norwegian Troll station revealed extremely low background concentrations, confirming minimal long-range inputs. In contrast, targeted sampling near wastewater outlets at one Antarctic research station showed clear evidence of local contamination in sediments, demonstrating the relevance of point sources in remote regions. Terrestrial matrices (moss, lichen, soil) showed almost exclusively non-detects, while krill showed exclusively non-detects.

In Svalbard, D4, D5, and D6 concentrations were higher than Antarctica, reflecting a combination of long-range or regional transport and local human activity. Air concentrations at the settlement of Ny-Ålesund exceeded those at Antarctic stations by two to three orders of magnitude, while a remote Arctic location (Zeppelin Observatory) showed low but detectable background levels. No or extremely low levels of the studied cVMS were detected in Arctic terrestrial and aquatic media, aligning with the Antarctic findings and supporting negligible accumulation in these matrices.

Together, these polar datasets provide the most geographically extensive assessment of D4, D5, and D6 in remote environments to date. The results highlight (i) the dominance of local emissions in shaping near-field concentrations at research stations, (ii) very low to undetectable levels in terrestrial and aquatic compartments, and (iii) clear hemispheric differences in atmospheric burdens. These findings contribute to ongoing regulatory discussions by demonstrating the extremely low environmental presence of the studied cVMS in pristine regions and the importance of distinguishing true background levels from isolated point sources.

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2.05.P-Mo125 Combined Effects of the Bio-pesticide Azadirachtin and Climate Change Warming Scenarios on Freshwater Communities

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Due to Global Climate Change (GCC), we expect rising temperatures and more often reoccurring heatwaves in aquatic ecosystems every year. Previous studies have found that warming may influence the toxicity of chemicals (e.g., pesticides) in the environment by complex mechanisms not yet fully understood. However, in standard ecotoxicological testing warming conditions are not usually considered. Pesticides commonly pollute aquatic ecosystems through spray-drift, agricultural runoff, and chemical spills. That is also the case for bio-pesticides, even if considered a more chemically-friendly option, their effects on non-target aquatic species might still be significant and are not well investigated. One of the most successful commercial biopesticides is

Azadirachtin. This Neem extract with Azadirachtin A (AzA) as the lead component is known for its strong insecticidal properties and its low persistence. Thus, it is key to evaluate its toxicity under different temperature regimes that reflect natural conditions with future climate change scenarios. The aim of this study was to assess the single and combined effects of the bio-pesticide NeemAzal-T/S (Neem product) and GCC warming scenarios (fluctuating elevated temperatures and extreme heatwave events) on freshwater communities under a realistic set up (outdoor mesocosms). The experiment consisted of a full factorial design with 9 treatments, combining 3 temperature scenarios (ambient, elevated temperature (+4°C) and recurring heatwaves (+8°C for 1-week intervals)) with 3 AzA concentrations (0, 30 and 300 µg/l) over a period of 6-weeks during late summer 2025. Direct and indirect effects on different trophic levels including zooplankton, macroinvertebrates, insects and macrophytes communities were evaluated. The main endpoints assessed included: biweekly samples of zooplankton, macroinvertebrates feeding rate and insect emergence; in-situ identifications of macroinvertebrates community; and macrophytes coverage. Preliminary results show clear effects from both AzA concentrations on the macroinvertebrates community and insect emergence. Combined effects of the two stressors are expected to be found on the zooplankton community; however, further analysis need to be conducted. The results from this study allows us to better understand the mechanistic pathways of these stressors' interactions for a more accurate risk assessment in the future.

2.05.P-Mo126 Developmental Hindrance Effects of Five Antifouling Agents on the Sea Urchin *Hemicentrotus pulcherrimus*

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Organotin compounds (e.g., TBT) were previously used as antifouling agents to prevent biofouling on marine structures. However, due to their harmful effects on marine life, their use has been banned internationally. As a result, various organometallic and non-metallic antifouling paints have been developed as alternatives. Nevertheless, these substitutes remain under continuous safety evaluation due to concerns regarding their biological hazards.

The aim of this study was to investigate the toxic and developmental inhibitory effects of selected antifouling agents (Chlorothalonil, DCOIT, Diuron, Dichlofluanid, and Irgarol) on the normal embryogenesis rate of the sea urchin *Hemicentrotus pulcherrimus*, a representative primary consumer in the marine ecosystem. Additionally, we sought to identify the effects of these substances on each stage of early embryonic development. Embryos exposed to the antifouling agents showed accelerated development during the 2.5–6 hour period. However, exposure to Diuron and Irgarol at half the effective concentration began to delay progression to the early gastrula stage after 18 hours, with significant developmental delays observed after 24 hours. Continued retardation was evident in the developmental sequence from early gastrula to gastrula, early pluteus, and pluteus stages. The severity of developmental delay followed the order: DCOIT > Irgarol > Diuron > Dichlofluanid > Chlorothalonil (with greater delay indicating lower developmental progression).

These findings suggest that the toxic effects of the five antifouling agents on sea urchins are largely attributable to disruptions in early developmental processes. Given that antifouling agents are highly persistent in the environment and are known to exert toxic effects on various marine organisms, including invertebrates as demonstrated in this study, it is critical to conduct further research at the genomic level. Moreover, the urgent establishment of effective environmental protection strategies is essential to prevent marine pollution and safeguard marine ecosystems.

Disclaimer/Disclosure:

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2.05.P-Mo127 Organic Pollutants Mobilization After the 2024 DANA Floods in Valencia (Spain): Implications for Environmental Risk

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Valencia province (Spain) suffered a catastrophic cut-off low (COL), known locally as DANA in October 2024, resulting in torrential rainfall and severe flooding. This extreme weather event generated approximately 2.24

million cubic meters of sludge, which, upon drying, formed a dense dust cloud containing particles potentially harmful to human health. Few studies have investigated the chemical fingerprint of sludge and dust residues left in the wake of COL events, especially for organic contaminants in Mediterranean regions. This study aimed to address this gap by characterizing the occurrence and distribution of both legacy and emerging pollutants in sludge and atmospheric dust following the DANA event. A total of 13 sludge and 16 dust samples were collected in November 2024 from the Valencia area, subject to mixed urban, industrial, and agricultural pressures. A broad spectrum of contaminants was analyzed, including pesticides, plastic additives (phthalates, non-phthalate plasticizers, and organophosphate esters), brominated flame retardants (BFRs), and short-chain chlorinated paraffins (SCCPs). Samples were analyzed using gas and liquid chromatography-tandem mass spectrometry. The obtained data were treated to perform an integrated environmental risk assessment (ERA). All major contaminant classes were detected, with total levels up to 9,000 ng/g wet weight (ww) in sludge and nearly 39,000 ng/g dry weight (dw) in dust. Sludge showed highlightable levels of legacy pesticides like endosulfan (max. 1,395 ng/g ww) and lindane (max. 363 ng/g ww). Dust contamination was dominated by plastic additives, specifically diisononyl phthalate (DINP) (max 13,117 ng/g dw) and di-2-ethylhexyl phthalate (DEHP) (max 10,286 ng/g dw). SCCPs were ubiquitous in dust (median of 620 ng/g dw). The ERA indicated a potential elevated risk (HQ>10) for 13 pesticides, 9 plastic additives, one BFR, and SCCPs in the worst-case scenario. The highest HQ (>10,000) corresponded to heptachlor epoxide, lindane, cypermethrin, and DEHP. The DANA flood acted as a significant mobilization mechanism for a wide spectrum of contaminants. The higher concentrations in dust suggest that dried sludge may act as a secondary pollution source and create a potential route for long-term human exposure. Characterizing this contamination profile is crucial for informing risk assessors and post-disaster remediation policies in vulnerable regions subject to increasing climate-related hydrometeorological events.

Disclaimer/Disclosure:

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2.05.P-Mo128 Sediment Toxicity in Northern Patagonia: Multiple Biomarker Responses in Gammarus spp.

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The Valcheta Stream, located in an endorheic basin in Northern Patagonia, Argentina, is a remote system increasingly exposed to diverse anthropogenic pollutants. To evaluate sediment-associated toxicity, we collected composite samples (right bank, left bank, and mid-channel) from three sites with different contamination sources—an agricultural impact zone, an urban-influenced area, and a sewage-affected reach—along with an upstream reference site. Lyophilized sediments were transported under cooled conditions to Germany and Switzerland, where we applied an integrated suite of physiological, behavioral, and molecular biomarkers using amphipods of the genus *Gammarus*.

Amphipods were collected from the Main River and acclimated for 5–10 days before exposure. Experiments followed OECD/ISO guidelines employing four sediment treatments and a negative control consisting of six replicates, six organisms per 250-ml aerated beaker at 16 °C (total 30 beakers, 180 organisms). Behavioral responses were measured at 24, 48, and 96 h with the ToxmateLab system (ViewPoint, Lyon), using alternating light–dark cycles. Automated analyses identified distinct activity patterns. Preliminary assessments of hyperactivity and hypoactivity revealed clear deviations from baseline locomotion in organisms exposed to sediments from the more impacted sites. Urban sites produced the greatest deviations, potentially suggesting a combination of stressors. Additional parameters, including activity time and average and maximum speed at light and dark conditions, are currently being evaluated in an attempt to obtain a higher sensitivity to these effects.

Following behavioral tests, organisms were frozen at –70 °C for subsequent biochemical and proteomic analyses. Planned endpoints include oxidative stress markers (lipid peroxidation via TBARS, antioxidant and

metabolic enzyme activities) and mass-spectrometry-based proteomics with label-free quantification on a Thermo Astral instrument operated in DIA mode. By integrating behavioral and molecular biomarkers, this study aims to establish sensitive early-warning indicators of sediment toxicity in the Valcheta Stream and to improve understanding of contaminant-related stressors in Patagonian freshwater ecosystems.

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2.05.P-Mo129 Beyond Single Stressors: A Review of Climate Modulation of Chemical Fate and Toxicity In Freshwater Ecosystems.

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Climate change is driving rapid and substantial changes in the frequency and intensity of extreme weather events. Global alterations to temperature, precipitation patterns, and acidification have the ability to dramatically disrupt the dynamic equilibrium of freshwater systems. These physicochemical changes are likely to influence chemical fate, behaviour, and effects so consideration of climate change should be integrated into the chemical risk assessment process. Previous reviews have explored how climate change can impact the fate, behaviour, and effects of chemical pollution, although recent advances necessitate an update to these reviews. This poster aims to synthesise these results on an endpoint-by-endpoint basis to best explore how climate change may impact species differently depending upon the study scale whilst simultaneously assessing how changes to chemical fate and behaviour may exacerbate this. Using a systematic review approach, a combination of 9 search strings and citation searching, 128 papers were identified. Over 68% of papers reviewed, suggested that climate driven changes are able to increase the toxicity of chemical pollutants; although, this result varied depending upon study design. Much of the literature was focused on temperature and pesticide pollution and considered survival and biochemical endpoints. Fewer than 10% of studies focus on climatic variables other than temperature (precipitation and acidification), many of which fail to be adequately assessed. This review has identified that these climatic variables can influence chemical fate, behaviour, and effects, impacting life-history, biochemical stress, and mortality. Whilst the evaluation of papers on effects on chemical fate is ongoing, preliminary results suggest that drought reduces dilution, flooding increases resuspension, whilst temperature and pH can significantly influence bioconcentration of pollutants. Further laboratory-based research is required to explore how extreme weather events influence an organism's physiology and energetics especially at the population and community level, whilst still accounting for biochemical and molecular endpoints. These results will be able to inform directions for further study, regarding multiple stressor studies in freshwater environments.

2.05.P-Mo130 Presence and distribution of microplastics in soils from the Arctic and Antarctic Peninsula

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In recent years, airborne microplastics have been identified in a range of remote environments including the Arctic and Antarctica. While most of the data available is focused in seawater and sediments, little is known about their present in Arctic and Antarctic soils. In this study, we have collected 20 surface soil samples from remote lakes in the Arctic and Antarctica during various sampling campaigns in 2024 and 2025.

Soil samples were homogenized and treated to isolate microplastic particles. Suspected microplastic particles were isolated and their composition confirmed using micro-Fourier transform infrared spectroscopy (μ FTIR). This study reports the identified microplastics in Antarctic and Arctic soils, being the highest in concentration, found in those soils collected close to research stations.

2.05.P-Mo131 Exploring the Fate of Tire-wear Particles and De-icing Salts in High Mountain Streams of Spanish Protected Areas

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As a consequence of the use of roads, particles are continuously being released due to the mechanical abrasion of tires with the road surface, which can enter nearby aquatic ecosystems through runoff water in protected areas, such as Spanish National Parks. In addition to rubber, these particles contain additives that are potentially toxic to aquatic communities, such as metals or polycyclic aromatic hydrocarbons (PAHs). In parallel, the use of de-icing agents for winter viability on roads in High Mountain National Parks could be causing an increase in the salinity of adjacent ecosystems. The objective of this work was to determine whether tire-wear particles (TWPs) and de-icing salts can enter streams of High Mountain National Parks from nearby roads. For this purpose, we collected water and sediment samples in rivers and streams with and without road influence in three National Parks in the north, centre and south of Spain during spring and fall 2025. We measured water quality parameters (temperature, dissolved oxygen, electrical conductivity and pH), ions (Cl⁻, Na⁺, Ca²⁺, Mg²⁺), metals (Cd, Cu, Cr, Ni, Pb, Zn), styrene-butadiene and polyisoprene rubbers, and different tire-derived additives, including PAHs, benzothiazoles, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) and its ozonated derivate, 6PPD-quinone. To assess major source points of TWPs and de-icing salts, we sampled road runoff water and adjacent soil in one of the parks during early spring. Only in one out of three roads sampled, a concentration of 2.04 µg/L styrene-butadiene rubber was detected in the runoff water. In the soil adjacent to this road, the concentration of zinc, often used as an indirect proxy of TWPs, was 1216 mg/L. In road runoff water, chloride and EC levels were significantly correlated and differed between the locations sampled. Chloride concentrations ranged from 1.2 mg/L to 30.7 mg/L, and EC levels ranged from 19.5 to 158.8 µS/cm. Chloride levels were significantly higher in streams influenced by roads with de-icing salt application, often showing tenfold differences. In stream water, rubber particles were not detected, and metals were generally below the limit of quantification. Our preliminary results suggest that de-icing salts may enter nearby aquatic ecosystems through road runoff, while TWPs and their associated additives may not reach streams from nearby roads and be potentially retained in the adjacent soil

Disclaimer/Disclosure:

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2.05.P-Mo132 Bioconcentration of metals in *Gambusia holbrooki* exposed to forest fire ash: implications for freshwater ecosystem risk assessment

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Rural fires can mobilise metals bound to ash, which may enter aquatic ecosystems through runoff, posing risks to freshwater organisms. These metals can persist in water bodies and accumulate in aquatic biota, potentially disrupting ecosystem health. This study investigated the bioconcentration of metals in the fish *Gambusia holbrooki* exposed to ash elutriates from a recent forest fire, under controlled laboratory conditions. Fish were exposed individually to 200 mL of elutriate, and ten replicates were running per sampling time. The test lasted 15 days, including 10 days of exposure and 5 days of depuration. The exposure medium was renewed every 48 hours. During exposure, water samples and ten fish were collected sequentially at 0, 12, 24, 48, 72, 96, 144, 192, and 240 hours, removing a set of ten containers at each time point. Following 240 hours of exposure, fish were transferred to uncontaminated medium for depuration, with sampling of ten fish at 12, 24, 48, 96, and 144 hours. Water parameters, including pH, conductivity, and metal concentrations, were monitored throughout. Metal accumulation in fish tissues was quantified using ICP-MS, and bioconcentration factors (BCFs) were calculated. Results showed rapid uptake of some metals, while depuration revealed partial elimination, with retention varying by metal. These findings demonstrate that wildfire ash can serve as a substantial source of metals to freshwater organisms, with bioaccumulation varying according to metal type, exposure duration, and depuration dynamics. As a pioneer, this study provides critical insights into post-fire metal behaviour in aquatic ecosystems, enhancing understanding of bioaccumulation under realistic exposure scenarios. These results can inform ecological risk assessments, guide monitoring strategies in fire-affected watersheds, and support management actions to mitigate metal contamination and safeguard freshwater biodiversity.

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2.05.P-Mo133 Elevated Mercury Concentrations in Riparian Predators Unaccounted for by Trophic Magnification

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Riparian predators integrate both nutrients and contaminants from neighboring aquatic systems through their predation of emerging aquatic insects. The reliance of some riparian taxa on aquatic subsidies has led to them being utilized as biosentinels of aquatic contamination, often justified through stable isotope studies ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and trophic magnification models. However, evidence suggests that both isotopic signatures and contaminant burdens can be altered during insect metamorphosis, which could propagate to riparian predators. Here, we measured total mercury and $\delta^{15}\text{N}$ in riparian and aquatic organisms in the headwater streams of the southern Appalachian Mountains (Tennessee, USA) to compare biomagnification in both systems. We found that biomagnification rates were similar, but that mercury concentrations in riparian organism were approximately 6x higher than neighboring aquatic organisms. We propose possible ecological mechanisms, occurring separately or simultaneously, that might explain these surprising observations: 1) metamorphosis bioamplifies mercury, 2) metamorphosis enriches $\delta^{15}\text{N}$ values, and/or 3) the fractionation of $\delta^{15}\text{N}$ that occurs during predation is less for the observed riparian predators than for the aquatic organisms. Further study is needed to elucidate the exact mechanism driving elevated mercury concentrations in riparian predators.

2.05.P-Mo134 Physically Based Pharmacokinetic (PBPK) model for the Arctic fish species Atlantic halibut (*Hippoglossus hippoglossus*)

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The reduction of sea ice in the Arctic has opened previously inaccessible areas to commercial activity, increasing anthropogenic pressure on this pristine environment. Climate change and rising emissions are introducing new substances into Arctic waters, threatening marine life and human consumers of fish. Due to the vast number of chemicals, it is not feasible to test all substances on all species, highlighting the need for predictive tools in environmental exposure, hazard and risk assessment. Physiologically based pharmacokinetic (PBPK) models are foreseen to play a key role in characterization of absorption, distribution, metabolism, and excretion (ADME) of pollutants and aid in vitro to in vivo extrapolation (IVIVE) in Next Generation Risk Assessment (NGRA).

This work aimed to support PBPK model development for a key Arctic species, the Atlantic halibut (*Hippoglossus hippoglossus*), by characterizing relevant physiological parameters. A multi-compartment PBPK model was established based on existing fish PBPK models. Each compartment was defined by volume, lipid and water content, and blood flow. Parameters were obtained through measurements in halibut and literature reviews. The data lays the foundation for Arctic-relevant PBPK models in general, a halibut PBPK in particular, and support future NGRA of chemical exposures to Arctic marine fish species.

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2.05.P-Mo135 Analysis of Mercury and Cyanide Concentrations in River Waters of Areas Within the Yanomami Indigenous Territory Affected by Illegal Gold Mining

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The rapid expansion of illegal gold mining in the Amazon and the associated use of mercury (Hg) and cyanide (CN⁻) have raised serious environmental and public health concerns for traditional communities within the Yanomami Indigenous Territory. In 2023, the Brazilian Institute of the Environment and Renewable Natural

Resources (IBAMA) and the Brazilian Center for Mineral Technology (CETEM) launched an Environmental Monitoring Network to assess contamination in multiple matrices within the Yanomami Territory and adjacent Conservation Units. Sampling points were chosen based on proximity to villages and mining sites, major rivers, and accessibility. Most areas are reachable only by small aircraft, imposing logistical challenges. This study presents results for total Hg and CN⁻ in surface waters. Over one year, 27 priority locations were monitored using standardized protocols for water, sediment, and fish sampling. Of 25 water samples analyzed for THg, 24 (96%) exceeded maximum permissible values. For CN⁻, 33 (63.5%) of 52 samples surpassed regulatory limits. Elevated CN⁻ near active mining suggests cyanidation processes, while high concentrations in Conservation Units with no mining history highlight the complexity of distinguishing natural sources from contamination. Reliable differentiation will require long-term monitoring, control sites, or advanced analytical methods. Historically, Hg dominated artisanal gold extraction due to amalgamation simplicity, but CN⁻ detection may indicate cyanidation, demanding stricter oversight. As sediment and biota data emerge and new campaigns proceed, understanding pollutant dynamics will improve—especially for cyanide, whose behavior in Amazonian systems remains poorly characterized. Despite limited baseline data, these findings represent a critical step toward establishing reference conditions and supporting policies to protect Indigenous and riverine communities from illegal mining impacts.

2.05.P-Mo136 Atmospheric deposition and long-range transport of micro- and nanoplastics

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Atmospheric deposition plays an important role in the global distribution and long-range transport of environmental pollutants towards remote regions. While modelling frameworks for long-range atmospheric transport are well-established for organic contaminants, mechanistic models for micro- and nanoplastics (MNPs) are limited. This challenges the interpretation monitoring data of MNPs in remote areas and our ability to estimate environmental exposure to MNPs at the global scale.

We here present a model framework for atmospheric transport of MNPs, with a particular focus on physical descriptions of deposition, which exerts the key control on atmospheric residence times and apply the framework to selected scenarios representing a wide range of MNP properties and environmental conditions, to disentangle their respective influences on the potential for MNP transport to remote regions. We combine existing equations and theories for atmospheric particle transport (e.g. from aerosol and pollen research) with empirical data on MNP properties (e.g. effect of surface ageing on hydrophobicity). We include multiple mechanistic descriptions of wet and dry deposition processes, accounting for particle size, shape, density, surface hydrophobicity) and the impact of land surface properties, surface wind speed, atmospheric stratification and precipitation patterns.

Our model framework enables an in-depth analysis of the different deposition mechanisms contributing to the atmospheric removal of MNPs. We estimate that atmospheric half-lives for MNPs can range from a few seconds to weeks and find that the main drivers for these differences are particle size and shape, precipitation characteristics, atmospheric stratification, land surface features and surface wind velocities. In all scenarios, deposition rates are lowest for intermediately sized MNPs (around 1 µm) and even lower for fibres, resulting in longest atmospheric half-lives. Under dry conditions, these MNPs can exhibit atmospheric half-lives of up to two weeks, which is sufficient for long-range atmospheric transport from Europe to the Arctic during weather patterns where prevailing air flows trajectories favor transport to high latitude.

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2.05.P-Mo137 Organofluorine in the Arctic and Northern European Atmosphere – a Current Overview

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The atmosphere is important for the global distribution of organofluorine contaminants (OFCs). Several are commonly known as per- and polyfluoroalkyl substances (PFAS), of which some pose a hazard for human and environmental health. Other OFCs, such as chlorofluorocarbons (CFCs), are ozone depleting substances (ODSs), and others, such as hydrofluorocarbons (HFCs), have high global warming potentials (GWPs) and

therefore contribute to ongoing climate change. OFCs are a vast group, and environmental persistence is a key common property for enabling their diverse range of hazards. Long-term atmospheric monitoring is important for understanding their sources and fate, as well as evaluating the effectiveness of international regulations such as the Montreal Protocol and Stockholm Convention. NILU monitors numerous OFCs in the atmosphere at three stations using four different measurement techniques. In this study, we measure 84 OFCs (containing at least one C–F bond) across three Norwegian sites: the Zeppelin Mountain (Arctic), Birkenes (rural Norway) and Oslo (urban) in 2023/24.

65 OFCs were detected at Zeppelin. This indicates the diversity of OFCs present in the background Arctic atmosphere. CFCs, HFCs, perfluorocarbons (PFCs) and halons were expectedly high (with mean concentrations 210 – 490,000 ppq). Several (semi-)volatile OFCs were lower – desflurane, perfluorotributylamine (PFTBA), perfluorotripentylamine (PFTPeA), tetrachlorohexafluorobutane (TCPFB) and perfluorophenanthrene (PFPHP), of which most were present at both Arctic and urban locations (0.50 – 360 ppq). Perfluoroalkyl acids (PFAAs) and their semivolatile precursors were lower still, with sum values often <1 ppq, but still notable given the bioaccumulative and toxic properties of some of these OFCs. Both PFAAs and their semivolatile precursors were higher at Birkenes and in Oslo than at Zeppelin, owing to closer proximity to polluted airmasses. Those compounds with the highest levels (CFCs, HFCs, PFCs and halons) are known to have long atmospheric lifetimes (often >10 years), and several are known ODSs and/or have high GWPs (often >1000).

The widespread detection of PFAAs and numerous other OFCs, including at a remote Arctic location, indicates the importance of the atmosphere for their global distribution. Long-term atmospheric monitoring is needed to understand their environmental processes and evaluate the effectiveness of their ever developing regional/international regulation.

2.05.P-Mo138 From the summit to the sea: Tracking biodiversity along interacting stressor gradients in a high-altitude tropical river

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Tropical high-altitude riverine systems, such as those found in the South American Andes, have a particularly high biodiversity potential, as they span a remarkable gradient of climatic and geological zones. As these rivers flow from their often-pristine headwaters through their middle reaches, ending up in lowland deltas and estuaries, their waters experience a highly diverse set of environmental variables. These variables can be natural or anthropogenic and can induce stress on riverine organisms. Naturally occurring stressors dictate niches and species composition along altitude gradients (Loayza-Muro et al., 2014). Alongside these natural stressors, human-impacted rivers often contain complex mixtures of micropollutants, which pose significant risks, including biodiversity loss, degradation of drinking water quality, and impairment of ecosystem services. However, little is known about the impacts of multiple interacting anthropogenic and naturally occurring stressors on river biodiversity, especially in tropical ecosystems.

Therefore, this project aimed to disentangle the effects of multiple interacting stressors on biodiversity in a tropical Andean river. The Rímac River is ±200 km long and flows from the highlands in the central Andes in Western Perú through the city of Lima to its mouth in the Pacific Ocean. The river flows from its source at approximately 4700 m down to sea level as it passes through increasingly anthropogenically impacted and urbanized landscapes, making it an excellent natural laboratory to study the impacts of multiple interacting stressors on river biodiversity.

The river was sampled at 15 locations along its course for physicochemical measurements, water, and sediment grab samples for nutrients, organic-, and inorganic pollutants. Macroinvertebrates and biofilm were collected for morphological and molecular biodiversity assessment.

The initial results show that macroinvertebrate biodiversity was lowest at both ends of the Rímac River, with very low numbers of individuals collected at the source in the urban zone of the river, and the highest diversity in the middle reach of the river. A sudden drop in biodiversity was observed starting at the city limit, coinciding with a strong influx of organic pollution. The results suggest that moderately anthropogenically impacted river sections can still host substantial biodiversity, while organic pollution in urban areas strongly impacts riverine biodiversity in the tropical Andes.

2.05.P-Mo139 Presence and distribution of legacy polychlorinated biphenyls (PCBs) and emerging Perfluoroalkyl substances (PFAS) in soil, freshwater and seawater from the Antarctic Peninsula

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Antarctic ecosystems, despite their remoteness, act as long-range sinks for persistent organic pollutants (POPs) both emerging perfluoroalkyl substances (PFAS) and legacy polychlorinated biphenyl (PCBs). Understanding the presence and distribution of these contaminants is essential as climate-driven melting may enhance the remobilisation of historically accumulated POPs. This study investigates the occurrence of PFAS and PCBs in freshwater and soils from Byers Peninsula (ASPA 126), as well as in seawater and soils collected at tourism-influenced coastal sites across the South Shetland Islands. Surface freshwater, seawater and soils samples were collected and analysed using solid phase extraction (SPE) cartridges followed by LC-MS/MS for PFAS. PCBs analysis required large-volume water sampling using XAD2 resin columns and subsequent GC-MS/MS quantification. Preliminary results reveal detectable PFAS across all matrices. Seawater near tourist landing sites showed PFAS ranging from 3.6 to 7.4 pg/L, while much lower concentrations were found in Byers' freshwater lakes. Long-chain PFCAs (notably PFDA and PFDaDA) and PFOS were the dominant compounds, reaching up to 1.9 pg/L. In soils, PFBA, PFOA and PFNA represented up to 60% of PFCAs. PCBs were also detected in soils and lake water, with higher concentrations observed during melting periods suggesting cryosphere release. These results provide the first integrated assessment of PFAS and PCBs across multiple Antarctic environmental compartments, offering baseline information to understand contaminant partitioning, persistence and potential links with tourism and climate-driven remobilization.

Disclaimer/Disclosure:

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2.06 Higher Tier Testing and Risk Assessment for Aquatic Organisms

2.06.T-01 A Deep Dive into Aquatic Mesocosms – Exploring Ecosystem Dynamics and Veterinary Drug Impacts

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In chemical risk assessment, standardized methods such as those by OECD are commonly applied to gain an impression of potential environmental hazards. However, extrapolating effects from these standardized experiments to broader ecosystem impacts can be challenging. Aquatic mesocosms are a well-established and valuable tool to bridge the gap to realistic exposure. But how can we further improve the data output from higher tier experiments supporting e.g. modelling and statistic interpretation?

The aims of the MeMo project were: to examine the year-round dynamics in aquatic model ecosystems, establish new endpoints, and provide a solid base data set for mesocosm modelling. Two mesocosm studies – one conducted under Good Laboratory Practice (GLP) standards – were carried out at a test facility at the Research-Centre Neu-Ulrichstein in Hesse, Germany.

Study A was designed to detect threshold levels of endectocide treated mesocosms. Each four replicates were treated once with different concentrations of the veterinary drug eprinomectin. The fate and ecotoxicological impact of the stressor was assessed over a span of 100 days post-application. Residues of the substance were measured in water, sediment and macrophytes. Despite a DT90 of approximately 50 days in the water phase, the drug was still detectable in plants and sediment after more than 80 days after application. In addition to abundance counts of zooplankton and macroinvertebrates and chlorophyll-a measurement, other non-lethal endpoints were assessed as well. These included calculating the mean time to emergence and sex ratio of emerging insects, as well as measuring the community structure of macroinvertebrates post-application. Concentration-dependent effects of up to 100% without recovery were observed in some groups, while clear indirect (e.g., higher abundances) concentration-response relationships were seen in others.

Study B focused on seasonal dynamics, population structure, and data translation in enclosure mesocosm systems. A mesocosm was sampled holistically on a monthly basis for one year. Population data were derived by standard sampling procedures and by the total abundance of the animals. This two data sets allow to generate a relation factor to link standard sampling to absolute abundances of the whole mesocosm for all species. At the

same time, all animals in the standard samples were measured to determine the seasonal progression in the population structure of aquatic organisms.

2.06.T-02 Long-term Effects of Nicotine on Freshwater Communities using Outdoor Mesocosms

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Nicotine and its main metabolite, cotinine, are released into the environment during the production and disposal of cigarettes, after the application of neonicotinoid pesticides or via wastewater discharges. Although nicotine and cotinine are widespread over the world's freshwater bodies, with concentrations reaching 11.7 µg/L and 9.57 µg/L, respectively, very limited data is available on their toxicity to aquatic species. This study aimed to assess the dissipation rate of nicotine and the formation of cotinine under semi-natural freshwater conditions and to assess their toxic effects to aquatic phytoplankton and invertebrate communities. A twelve-weeks mesocosm experiment was conducted using outdoor 1 m³ circular tanks, which included 4 untreated controls and 5 exposure concentrations (0.1, 1, 10, 50 and 100 µg/L), in triplicate. On days 0, 28, 56 and 84, water samples were taken from all mesocosms for nicotine and cotinine analysis. Weekly water samples were collected in two treatments to assess nicotine degradation and cotinine formation. Water quality parameters were monitored weekly, and phytoplankton, zooplankton and macroinvertebrate samples were collected on days -7, 14, 28, 56, and 84. Emergence traps were installed to collect insect adults emerging from the mesocosms over time. Total average nicotine concentrations through the whole exposure period were 0.17, 0.69, 7.91, 32.5, and 84.3 µg/L for the nominal concentrations of 0.1-100 µg/L, respectively. A significant positive correlation between the degradation of nicotine and the air temperature was found. Principal Response Curve analysis showed no significant effects of nicotine on the different communities, except for zooplankton. The taxa that showed consistent treatment-related effects were *Daphnia pulex*, Ostracoda, *Keratella tropica*, *Echinogammarus* sp. and Lumbriculidae. The first three species had very low population abundances, making it difficult to establish robust conclusions. In contrast, *Echinogammarus* sp. and Lumbriculidae showed a consistent treatment-related population decline and had higher abundances, with the lowest population No Observed Effect Concentration (NOEC) being 10 µg/L. Our results indicate that nicotine does not pose consistent effects on the aquatic community structure at the tested concentrations. However, considering the lowest population NOEC identified in this study, risks could occur at hotspot locations for two of the macroinvertebrate populations studied.

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2.06.T-03 Comparing Morphological and Molecular Diatom Identification in a Multi-Stressor Microcosm Experiment

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Protecting aquatic ecosystems from pesticide pollution is a critical regulatory task. Benthic diatoms, as part of aquatic biofilms, are commonly used to indicate nutrient pollution. To assess if they could also serve as bioindicators for pesticide pollution, we conducted a laboratory experiment using flow-through chambers for aquatic biofilm colonisation and exposure, integrating realistic gradients of pesticides, light, temperature, and nutrients to optimise transferability to the field.

Diatom community composition can be analysed using both morphological and molecular techniques. Currently, it is unknown how these two methods compare in their ability to detect pesticide-responsive taxa. This study therefore evaluates whether morphological and molecular identification diverge in their detection of diatom species that respond to realistic pesticide mixtures. For both identification techniques (morphological and molecular), no significant differences in community composition based on relative abundances were detected at the genus level. At the species level, a significant

difference in diatom community composition was observed between taxonomic experts' identification, driven primarily by morphologically similar groups where subjective interpretation affects species resolution. For the molecular data, species-level assignments were higher with the older curated database version than with the more recent one, suggesting that increased curation reveals taxonomic uncertainty previously masked.

Significant discrepancies between identification techniques were detected at both the genus and species levels, likely reflecting methodological differences such as size-related detection thresholds in microscopy and abundance biases inherent to amplicon sequencing. Despite these differences, beta-regression analyses indicated that most species exhibited convergent patterns across methods, suggesting that the overarching ecological signal remains robust even when taxonomic resolution varies.

With this study, we highlight how taxonomic assignment discrepancies propagate into ecological interpretation and demonstrate how methodological harmonisation and curated reference databases can strengthen comparability between approaches. These findings offer a systematic evaluation of the suitability of next-generation sequencing alongside traditional morphology-based approaches and contribute to advancing diatom monitoring frameworks for assessing pesticide impacts.

2.06.T-04 Bridging Lab Studies to Mesocosms: Case study for *Cloeon dipterum* to Understand Temperature Mediated Effects of Imidacloprid Exposure

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Temperature can play an important role in shaping the effects of chemical stress responses, leading to shifts in study outcomes. Evidence from non-GLP (good laboratory practice) mesocosm studies for neonicotinoids (e.g., imidacloprid) suggests that warmer environmental conditions can shift the sensitivity of organisms and the resulting ecological endpoints. Risk assessment endpoints derived from laboratory studies are typically conducted at standardized optimal food and temperature conditions to measure the effects of the test item with high precision. To bridge this gap, we apply a mechanistic population model with temperature-dependent TKTD (toxicokinetic-toxicodynamic) processes, including lethal and sublethal effects such as immobility and reduced feeding.

Individual life histories of *Cloeon dipterum* are simulated with a Dynamic Energy Budget (DEB) model calibrated using variable-temperature laboratory data and fed into a population model. The population model is evaluated against available mesocosm experiments conducted across tropical, subtropical, and Mediterranean climates. Across climates, observed effects in mesocosms aligned with model predictions derived from laboratory calibration. Differences in mesocosm endpoints were attributable to a combination of extended exposure duration, temperature, and poor food availability. No additional adverse mechanisms were required to fit the observations in mesocosms beyond those established in laboratory studies. Notably, recovery in mesocosms often exceeded model predictions.

Our findings demonstrate that mechanistic population models, when properly calibrated on variable-temperature laboratory data, can reliably predict mesocosm outcomes across diverse environments, supporting their use as tools in environmental risk assessment.

2.06.T-05 Predictability of higher-tier outcomes in the aquatic risk assessment of plant protection products: Evidence from EU regulatory data

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The environmental risk assessment of plant protection products (PPPs) in the European Union uses a tiered framework where conservative Tier 1 estimates can be refined through higher-tier modelling or field studies. However, it remains uncertain how well Tier 1 risk quotients (RQs) predict outcomes in subsequent tiers, and whether refinement systematically reduces risk. Gaining this understanding can support more effective regulatory decision-making and help identify cases where further refinement is either justified or unlikely to alter the final conclusion.

To address this, we analysed a regulatory dataset covering 676 aquatic Tier 1 assessments for EU-approved insecticides and acaricides. For each substance–use–organism combination, we recorded the Tier, FOCUS step, organism group, time scale, and RQs. We then identified the first tier or step achieving acceptable risk (RQ ≤ 1), quantified reduction factors between Tier 1 and the first acceptable refinement level, and applied ordinal logistic regression and decision tree models to evaluate whether Tier 1 exceedance magnitude predicts refinement outcomes.

Most Tier 1 assessments (781%) surpassed the acceptability threshold. Among these, about half achieved

acceptability within Tier 1 refinements, while approximately 40% remained unacceptable at all refinement levels. Reduction factors consistently increased with each refinement level, typically gaining one to two log-units per tier. Model results indicated that the magnitude of Tier 1 exceedance strongly predicted the extent of required refinement. The decision tree identified two thresholds in Tier 1 log₁₀ RQ—about 1.7 and 2.9—that distinguished cases usually resolved at Tier 1, those with mixed outcomes, and those that needed Tier 3 or remained unacceptable. Many “no acceptable” outcomes reflected a lack of submitted higher-tier studies rather than confirmed ecological risk.

Overall, the analysis shows clear quantitative links between Tier 1 exceedances and the success of refinement. These insights may help develop benchmarking tools and prioritisation schemes to make future PPP evaluations more efficient. Ongoing work will extend this analysis to herbicides, plant growth regulators, and fungicides to further test the broad applicability and regulatory significance of these findings.

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2.06.P Higher Tier Testing and Risk Assessment for Aquatic Organisms

2.06.P-Th005 Challenges in aquatic higher tier risk assessment for pesticides

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Aquatic organisms can potentially be exposed to plant protection products, biocides and general chemicals via numerous exposure routes. Taking the example for plant protection products, there is a potential for exposure to edge of field water bodies, and thus a risk assessment is required under Regulation (EC) 1107/2009.

The current guidance document used for the aquatic risk assessment of Plant Protection Products (AGD) in the European Union (and GB) was adopted in 2013. Advancements in regulatory science since then have created an opportunity to revisit elements of the guidance that would support the adoption of a more efficient approach to aquatic risk assessment.

For example, mesocosm studies and higher tier studies in general, are a challenge due to their complexity (black box problem), their high amount of data and less well-defined evaluation criteria when compared to standardised laboratory studies, presenting issues around subjectivity. So, there are still some open issues and uncertainties how higher tier studies should be conducted, reported, evaluated and used.

In this poster, the authors will present some of their concerns around common higher tier approaches for aquatic risk assessment.

One example of open issues is the use of Minimum Detectable Difference for mesocosm studies. Reporting was required by EFSA PPR panel (2013) but due to the missing experience there was no clear guidance on how they should be used. Thus, industry and test facilities need to know better what regulators expect. On the other hand, it becomes more difficult for the notifiers to decide whether resources should be spent into such complex studies due to uncertainties on requirements in the coming years. In addition some member states are considering simplifying the tiered approach and to avoid higher tier studies in general. This in turn leads to the risk that effects at the population level and indirect effects at the community level are completely ignored.

The purpose of this poster is to stimulate a joined-up discussion on aquatic higher tier risk assessment with the goal of establishing a SETAC tripartite interest group to support the future development of updates to the aquatic guidance document.

2.06.P-Th006 Risk assessment of plant protection products (PPP) for aquatic organisms: relevance of exposure pattern in mesocosm studies compared to predicted exposure in surface waters

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For the authorization of plant protection products (PPP) in the context of the Regulation (EC) N°1107/2009, a higher tier risk assessment for aquatic organisms can rely on mesocosm studies. According to the “Guidance on

tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters” (EFSA Journal 2013;11(7):3290), the exposure pattern in a mesocosm study should be realistic to worst case compared to the relevant predicted exposure profiles in edge-of-field surface waters based on FOCUS methodology (FOCUS (2001). “FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC”. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp). Such comparison is also called “concept of the envelope curve” in the Critical Appraisal Tools (CATs) (Proposal of critical appraisal tools for the evaluation of ecotoxicology studies, EFSA Supporting publication 2023:EN-7787) developed for mesocosm studies. For this purpose, “Regulatory Acceptable Concentration profiles” (“RAC profiles”) should be generated and plotted alongside the predicted exposure profile in edge-of-field surface waters. Some critical issues have been raised in recent years during peer review of active substances and PPP concerning the relevance of exposure pattern in mesocosm compared to predicted exposure profiles (e.g., acceptability of exposure pattern in mesocosm for the intended uses, method to be used to show the “concept of the envelope curve”, ecotoxicological dependence/independence of the peaks, etc.). The aim of this poster is to show the usefulness of graphical illustration to address these issues, through some recommendations and few illustrative examples. Overall, this approach facilitates the interpretation of results and supports their use in risk assessment of plant protection products.

2.06.P-Th007 The Role of Mesocosm Data in Ecotoxicological Legislation

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Mesocosm studies serve as a regulatory accepted study, seen as bridging a gap between lower tier laboratory-based toxicity tests and dynamic natural ecosystems. They represent standardised experimental systems that aim to capture the interactions, variability, and recovery dynamics of aquatic communities and populations under realistic environmental conditions. Within the context of environmental risk assessment for plant protection products (PPPs), mesocosms provide higher-tier data that go beyond individual species response, generating ecologically relevant data into population, community, and functional-level effects.

In regulatory frameworks, mesocosm studies can be used to simulate real-world exposure scenarios while maintaining reproducibility and scientific consistency. This balance between control and environmental realism enables authorities, including the European Food Safety Authority (EFSA), to integrate mesocosms as refinement options for aquatic risk assessments. These studies can also help to define the environmental safety thresholds used in pesticide registration and management.

Multivariate statistical approaches, such as Principal Response Curves (PRCs), offer a multidimensional perspective on ecological integrity. They allow for an assessment on both immediate toxic effects, and on recovery following exposure. Understanding how these endpoints are interpreted within regulatory evaluations is key to determining how mesocosm data influence final legislative outcomes.

However, the complexity of mesocosm datasets also presents challenges for interpretation across studies and the integration of mesocosms into regulatory decision-making. Whilst there may be efforts toward standardisation in an aim to increase comparability and transparency across studies, differences in design that are required for chemical loading and behaviour, sediment characteristics, species composition, and environmental conditions can all affect study outcomes.

The aim of this poster is to show how mesocosm experimental data can inform the development, refinement, and implementation of environmental legislation related to PPPs. By linking practical experimental evidence to policy frameworks, mesocosm research has the potential to strengthen the scientific foundation of ecotoxicological regulation, giving confidence in environmental protection goals, and ensuring that legal standards are grounded in ecological realism.

2.06.P-Th008 Sediment: an undervalued compartment in mesocosm studies

Ellie Welch, **Charlotte Court** and **Nadine S Taylor**, Cambridge Environmental Assessments (CEA), United Kingdom

The sediment layer is a key part of any mesocosm test system, however, its influence on the test design is largely undervalued. Generally, the sediment compartment is only of interest if the test substance is known to have physio-chemical properties of concern, e.g. they would bind to the sediment, or degrade slowly, and is often only investigated for analytical residues. However, the sediment composition can influence the biological community and the interpretation of mesocosm studies. Variations in sediment type, from sandy and silty/clay substrates, to the amount of organic matter present, can alter chemical movement, nutrient cycling, biological interactions, and function of benthic habitats within test systems.

Physical characterisation of sediment is usually focused on the percentage of stones > 10 mm to interpret the

sediment structure. Whereby, variability in the coarseness of the sediment affects, macrophyte health, filtration, and groundwater exchange. In these aspects sandy based sediments, with little to no organic matter, are considered to represent a “worst-case” scenario for retention of water and ease of transport. Dipwell monitoring of groundwater simulations could be valuable data to interpret contaminant dynamics for transport and exposure persistence, thereby influencing test system stability and recovery. This could be carried out with a stone or mesh base with an overlaying membrane and a siphon tube.

Varying sediment types would also influence the biological impacts within the mesocosm, e.g. through activities of burrowing macroinvertebrates and macrophyte stability, highlighting how biological disturbance can alter sediment-water interactions and exposure profiles.

The aim is to highlight the need for sediment-type considerations in the design and interpretation of mesocosm studies. Moving forward, there is a potential to create an ‘ideal’ sediment that incorporates more sand or clay in their design according to what is most appropriate, incorporation of groundwater monitoring to understand transportation dynamics, and monitoring macrophyte health as a proxy for sediment structure and function. Knowing the influence of sediment structure on contaminant transport and exposure dynamics enhances ecological understanding and data interpretation of mesocosm studies. The influence of sediment characteristics on the chemical, physical and biological function of the test system should have greater consideration in mesocosm studies.

2.06.P-Th009 Considerations for Designing Emergent Insect Traps in Mesocosm studies

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The emergence of insects from aquatic habitats is a key indicator of ecological integrity and reflects how successfully aquatic insects complete their life cycle within a test system. As such, it provides an integrated signal of population dynamics, contamination effects, and overall ecosystem function. These taxa are known to be sensitive to chemical stressors and are considered a key biological compartment in mesocosm studies. Reliable, representative emergence data are essential for assessing impacts on community structure and function.

Trap design plays a major role in the accuracy and representativeness of these measurements. Different insect groups use different emergence routes; some emerge directly from the water surface, while others climb macrophytes, or use the pond edge. Deployment of a single trap, or even a single trap type, may, therefore, under-represent important taxa. Effective traps must consider emergence behavior, placement within the mesocosm, mesh characteristics, and accessibility for sampling activities.

This poster presents a theoretical comparison of potential trap designs suitable for regulatory mesocosm studies. The design challenge is to balance two goals; (1) allow natural ecosystem functioning, including recolonisation, free movement of insects and population recovery, and (2); capture a sufficiently representative proportion of emergent organisms to support reliable trend detection and statistical analysis.

The baseline design is a traditional cone-shaped emergence trap with a pitfall trap on top. This design is well established, but primarily only captures taxa emerging directly from the water surface. It offers limited support for species dependent on plant surfaces or solid substrates.

Alternative designs considered here include cone traps using artificial macrophytes to improve representation of vegetation-dependent emergers; plant basket traps enclosed with mesh and topped with a pitfall collector, targeting taxa emerging from submerged or emergent plants and full-coverage mesh “roof” systems with pitfall traps to catch both vertical and horizontal dispersers.

Each design is compared based on coverage of emergence pathways, capture efficiency, ease of deployment, consistency across replicates, and suitability for requirements. The objective is to identify trap configurations that improve representativeness without compromising mesocosm data integrity and ecosystem function.

2.06.P-Th010 Emerging Evidence: The fungicide pyraclostrobin reduces aquatic insect emergence

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Pesticide emittance is seen as one of the leading causes of global declines in insect biomass, which has repeatedly been shown over the last few decades. A decline of, specifically, emerging aquatic insects may directly limit energy flow from aquatic to terrestrial systems, altering ecosystem structure, and impeding ecosystem functioning, and services. Although some research has examined how insecticides affect insect emergence in natural populations, very few studies have explored the effects of other pesticide classes – such as fungicides. In addition, ecotoxicity data for fungicides are scarce for many groups of emerging insects.

In this study, we investigate the impact of environmentally relevant concentrations of the strobilurin fungicide

pyraclostrobin on insect emergence, and their aquatic larvae, for the most common emergent insect orders (i.e. Diptera, Coleoptera, Ephemeroptera, Odonata, and Trichoptera). We applied a field-realistic concentration range (based on data analyses of the Dutch Pesticide Atlas) of pyraclostrobin (concentration range up to 29 µg/L) to 32 experimental ditches, representing a non-target small-bodied freshwater system closely linked to agricultural land. The ditches were naturally colonized prior to the start of the experiment by communities from the adjoining canal, leading to a robust and realistic insect community within our experimental set-up, allowing for the assessment of causal toxicity effects on emerging insects. We tracked insect emergence two months prior, and three months post treatment application. Additionally, we aim to determine toxicity data (i.e. effective concentration values) from the observed biomass and abundance per insect order. We observed a decline in emerged insect biomass and abundance with increasing pyraclostrobin concentration for four out of five common insect orders, with an effective concentration of 10% reduction in total insect biomass at 2 µg/L. This decline in emergence could not directly be related to a decline in aquatic insect larvae. The data are currently being analyzed and prepared for scientific publication, and will be presented at SETAC 2026 upon acceptance of this abstract.

2.06.P-Th011 The Right Species Matter: Advancing Macroinvertebrate and Zooplankton-Based Species Sensitivity Distribution Studies Through Targeted Selection and Cultivation

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Accurate Species Sensitivity Distribution (SSD) modelling relies heavily on the ecological relevance and experimental reliability of organisms selected for laboratory investigations. Freshwater macroinvertebrates and zooplankton as key indicators of ecosystem health, offer strong potential for linking field observations with controlled experimental data.

This project establishes a systematic framework for selecting suitable macroinvertebrate and zooplankton species for SSD development, integrating ecological traits, habitat specificity, tolerance characteristics, and the practical feasibility of laboratory cultivation.

The resulting shortlist of selected species, combined with an exemplary SSD analysis, demonstrates how careful test-species selection underpins realistic and ecologically meaningful risk assessments. This approach strengthens the protection of sensitive and conservation-relevant freshwater taxa and supports more robust regulatory decision-making in ecological risk assessment.

2.06.P-Th012 Systematic and objective profile analysis in support of use of an ERO-RAC (ecological recovery option-regulatory acceptable concentration) from a mesocosm study: a case study with lambda-cyhalothrin

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Experimental ecosystem (microcosm/mesocosm) studies are a valuable refinement tool in aquatic risk assessment for plant protection products (PPPs), accommodating environmental fate and behaviour of the active substance while addressing ecological processes such as community level effects and recovery. The latter is addressed by recommendations in the Aquatic Guidance Document (AGD, 2013) on derivation of regulatory acceptable concentrations (RACs). Both ecological threshold option (ETO) and ecological recovery option (ERO) RACs may be derived from a mesocosm if the recovery meets certain criteria and the exposure profile in the study is representative of those expected in edge of field surface waters. Additional guidance was released by EFSA as part of the Critical Appraisal Tool (CAT) for relevance of mesocosm studies (2023), which builds on the AGD in proposing the “concept of the envelope curve”: that a RAC profile be generated by dividing each measured concentration of the mesocosm exposure pattern at the relevant treatment level (the NOEAEC) by an assessment factor throughout the measured mesocosm profile. However, the practicality of checking that this RAC profile fully ‘covers’ the exposure profiles generated in FOCUS depends greatly on the complexity of the predicted profiles, in turn dependent on the physicochemical properties of the active substance, the intended uses and the local conditions. In response to a request from a Member State for analysis of many profiles according to the CAT for intended uses of a lambda-cyhalothrin product (KarateTM), we developed a methodology for systematic and objective analysis in accordance with the “concept of the envelope curve”. This comprised identification of toxicologically independent clusters of peaks, visual assessment of the clusters relative to the RAC profile, and where necessary detailed assessment of peaks using a “Profile Comparison Factor” (PCF). The PCF provides a numerical integration of key attributes of a cluster of peaks that exceed a threshold (ETO-RAC), and indicates the intensity of exposure to an active substance in a cluster of peaks in a

FOCUS profile, relative to the exposure profile in the mesocosm study, by reference to the following parameters: number of peaks, total duration of cluster (from start of first exceedance to end of last exceedance), maximum duration of a single exceedance in the cluster, total duration of intervals (exposure below ETO-RAC) within the cluster.

2.06.P-Th013 Evaluation of mesocosm studies conducted in support of higher-tier aquatic risk assessment – look what the CAT dragged in

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Experimental ecosystem (mesocosm/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 uses of PPPs. In spite of the obvious value of mesocosm studies in higher tier aquatic risk assessment, their acceptance by regulators is still subject to significant uncertainty. This relates both to technical conduct of the studies, in particular statistical power and taxonomic representation, but also to the ability to use a recovery endpoint. Use of an ecological recovery endpoints is increasingly contingent on the ability to demonstrate that the exposure profile generated in the mesocosm study is sufficiently worst-case. In 2023 EFSA published their “Proposal of critical appraisal tools (CATs) for the evaluation of ecotoxicology studies” and the CAT for evaluation of mesocosms provided additional guidance on profile analysis, namely the “concept of the envelope curve”. This appears to present a more conservative approach to assessing the representativeness of mesocosm exposure profiles. While a specific checklist directly addressing the characteristics of mesocosm studies is in many ways preferable to trying to use a generic approach, e.g. Klimish score, it seems worthwhile to compare this tool with the pre-existing guidance (Aquatic Guidance Document, 2013) and the checklist for assigning a Reliability Index therein, to see whether coverage of key aspects is comparable and the level of conservatism has been maintained. We found that the wording of the items in the CAT is rather brief and in places ambiguous, which potentially leads to uncertainty as to how it will be applied. Interestingly, there are more items in the CAT referring to the mode of action of the test substance than in the 2013 guidance while there is less attention to basic aspects of ensuring reliable exposure. Realism of exposure is given more prominence in the CAT but little practical detail is provided on how to adequately demonstrate representativeness of exposure profiles using the concept of the envelope curve, which appears to be a more conservative approach for anything other than the simplest expected profiles.

2.06.P-Th014 Revisiting Time-Weighted Average Approaches in Aquatic Risk Assessment: Scientific Basis, Regulatory Challenges, and Future Direction

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The EFSA aquatic guidance (2013) introduced the use of Predicted Environmental Concentration Time-Weighted Average (PEC_{sw;twa}) in chronic risk assessment under specific conditions, primarily when PEC_{sw};max exceeds RAC_{sw};ch. This approach is based on the principle of reciprocity (Haber’s law), which assumes toxicity depends on both concentration and exposure duration, allowing similar effects from short-term high concentrations or long-term low concentrations. EFSA guidance requires evidence such as early treatment-related responses, minimal latency of effects, and overlap with sensitive life stages of short duration. A case where PEC_{sw;twa} was successfully applied has been documented (Belgers et al., 2011), demonstrating its potential scientific validity.

However, EFSA’s 2015 technical report highlighted ambiguities and stressed that linear reciprocity must be demonstrated experimentally before PEC_{sw;twa} can be used. It also clarified latency considerations: gradual effect progression may allow PEC_{sw;twa} use, whereas late-onset effects indicate latency and require additional testing. Higher-tier refinements using TWA approaches remain rare due to lack of standardized guidance, reluctance among evaluators, and uncertainty in practical implementation.

Given that EFSA aquatic guidance does not foresee revisions soon, this project aims to re-evaluate PEC_{sw;twa} applicability. Specifically, it will:

- Critically analyze objections raised in recent EU active substance renewals.
- Examine uncertainties and shortcomings in applicant data and argumentation.
- Address key issues from EFSA (2015), including defining time frames for reciprocity, reporting endpoints when reciprocity applies partially, and clarifying latency assessment.

Case studies on data-rich substances will illustrate common pitfalls and propose solutions. Scientific evidence supporting reciprocity will be explored, particularly for herbicides used in aquatic weed control, where this

concept is essential. Ultimately, the project seeks to provide recommendations for harmonizing guidance, improving transparency, and enabling scientifically robust higher tier refinements in aquatic risk assessment.

2.06.P-Th015 Be more realistic: Predicting the effects of short-term exposure events on Zebrafish Early Life Stages using the GUTS-model

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Toxicokinetic-toxicodynamic (TKTD) models are increasingly used to analyse and predict effects of time-variable exposure on survival and sublethal endpoints. Here we present an example on the use of the GUTS model to describe effects of an insecticide on survival of fish larvae. In a Fish Full Life Cycle Test with zebrafish under flow-through conditions to achieve constant exposure, survival of larvae of the parental and filial generation was the most sensitive endpoint. Since results for both generations did not differ significantly, the pooled data were used to calibrate the reduced GUTS model by means of the openGUTS software. Both, the stochastic death (SD) and the individual tolerance (IT) variants of the reduced GUTS model could be fitted well to this data set. To validate the model, a zebrafish Early Life Stage test over 35 d was designed to provide data on effects of short-term exposure as recommended by EFSA's Scientific Opinion on TKTD modelling (EFSA PPR Panel 2018). The exposure pattern was decided based on available FOCUS exposure estimations and practical considerations. Effects of three exposure concentrations, each resulted from two pulse exposure events of 24 h and an interval of 7 days in between, were tested. The pulses were on day 7 and 15 post fertilization. A second larger interval to test toxicologically independent peaks was not tested because independent peaks would require an interval exceeding the duration of the test. Animal welfare is a further strong argument to skip testing of a second interval. The reduced GUTS model was able to predict the survival over time observed in the pulse exposure experiment reasonable well. Since the model validation showed acceptable goodness of fit parameters, we conclude that the model can be used as a Tier 2C approach to assess effects of the diverse dynamic exposure pattern predicted by FOCUS modelling on the survival of zebrafish larvae.

2.06.P-Th016 Exploring Climate-Linked Shifts in Zooplankton Communities and Water Quality Parameters Using Historical Mesocosm Data

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Mesocosm studies can provide valuable insights into how aquatic ecosystems respond to environmental stressors under controlled, yet ecologically relevant, conditions. Mesocosm facilities typically have an abundance of historical data from these studies that has the potential to be utilised to investigate and interpret long-term trends and changes. In this poster, we analysed a unique archive of historical mesocosm data, spanning a decade, from Cambridge Environmental Assessments (CEA); a GLP-compliant facility in Cambridgeshire, UK, specialising in the aquatic testing of plant protection products (PPPs). The data was used to investigate potential climate-driven trends in water quality parameters and zooplankton dynamics, with a particular focus on changes in zooplankton communities.

Although designed for higher-tier regulatory assessments, the consistent experimental protocols, semi-natural conditions, and detailed monitoring at CEA provide a unique opportunity to explore long-term ecological patterns. We analysed trends in baseline water quality parameters (e.g., temperature, dissolved oxygen, pH, and conductivity), alongside zooplankton community structure, abundance, and diversity.

Preliminary findings indicate subtle, but consistent, shifts in zooplankton community structure and behaviour over time. These outcomes emphasise the value of long-term mesocosm data for detecting ecological signals of climate change and highlight the need for further research into how global change may influence intra- and interspecific community structure and function in aquatic systems.

These findings reveal that historical GLP mesocosm data, such as those from CEA, could provide valuable insights into the subtle ecological impacts of climate change. Beyond their regulatory function, such datasets may serve as important archives for understanding how global change influences freshwater ecosystems and species interactions over time.

2.06.P-Th017 Ecotoxicity of agricultural pesticide mixtures in freshwater microcosms: Insights into bioconcentration and trophic transfer

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Freshwater ecosystems near agricultural areas are highly vulnerable to complex pesticide mixtures that can affect aquatic organisms. Predicting organismal responses to simultaneous exposure to multiple pesticides remains a major challenge in ecotoxicological risk assessment. Within the H2020 SPRINT project, we addressed this challenge using a high-tier evaluation based on a two-trophic-level aquatic microcosm structured into three experimental stages. The microcosm included a producer (*Nasturtium officinale*), a primary consumer (*Atyaephyra desmarestii*), and a secondary consumer (*Procambarus clarkii*). Two pesticide mixtures, each containing five active substances detected in aquatic systems of Spain and Czechia, were tested under control conditions and at predicted environmental concentrations (PEC). Results showed that pesticide levels in producer tissues directly determined the amounts ingested by the primary consumer. The highest bioconcentration occurred when the primary consumer ingested contaminated producers while simultaneously being exposed to PEC, underscoring the combined influence of dietary and waterborne exposure. Bioconcentration factors varied regionally: acetamiprid showed the highest accumulation in Spain, whereas terbutylazine had the highest, though lower overall, accumulation in Czechia. Biomagnification factors were consistently low across both case studies, indicating limited trophic transfer from *A. desmarestii* to *P. clarkii*. Overall, the study indicates a low risk of bioaccumulation and biomagnification of the tested pesticide mixtures within this simplified aquatic food chain. These findings are ecologically encouraging yet highlight that pesticide behaviour varies across regions, warranting further investigation. The study advances ecotoxicological assessment by providing an experimentally validated approach for evaluating pesticide mixtures under realistic exposure scenarios and offers insights that support more accurate environmental risk assessments.

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2.06.P-Th018 Ecological Risk Assessment of Growth and Reproductive Toxicity of Benzalkonium Chloride in Aquatic Environments

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Benzalkonium chloride (BAC), a quaternary ammonium cationic surfactant, is widely used as a disinfectant and biocidal agent in environmental and household applications. Following the surge in disinfection demand during the SARS-CoV-2 pandemic, BAC usage has increased substantially, with residues potentially entering aquatic environments through domestic sewage and hospital wastewater. Previous studies have detected BAC in environmental waters—for instance, 31.4 µg/L in municipal wastewater in the Netherlands and 256.6 µg/L in Poland—indicating potential ecological risks that require further investigation. In this study, the model organism *Caenorhabditis elegans* (*C. elegans*), commonly used in environmental toxicology research, was employed to evaluate the ecological toxicity of BAC, using the Love River in Kaohsiung City, Taiwan, as a representative urban river system. The maximum BAC concentration detected in the Love River was 35.2 µg/L. Toxicity assessment revealed that BAC inhibited the growth and reproduction of *C. elegans* and increased intracellular oxidative stress. The predicted no-effect concentration (PNEC), estimated using the Hill model, was approximately 0.033 µg/L. Furthermore, exposure experiments at the highest concentration (1000 µg/L) demonstrated that reproductive toxicity induced by BAC was positively correlated with total nitrogen and total phosphorus levels in river water samples. These findings suggest that BAC in urban river systems may pose

potential ecological risks, and the results of this study provide valuable reference information for the ecological safety assessment and environmental management of BAC and similar disinfectants.

2.06.P-Th019 Interactive Effects of Light Pollution and Co-occurring Stressors on an Aquatic Insect (*Chironomus riparius*)

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Light pollution or the excessive use of Artificial Light at Night (ALAN) negatively affects most urban aquatic ecosystems with potential negative ecological consequences for aquatic biota. The highest levels of ALAN occur in urban areas, where aquatic ecosystems are also exposed to other stressors such as warming, salinisation and pesticide exposure. These stressors can interact and act additively or even synergistically. However, combined stressor effects with ALAN are still unexplored.

In this work, we evaluate the effects of light pollution (1 and 10 lux) with increased temperature, salinity and pesticides (glyphosate) on the physiological and life-history responses of the non-target species *Chironomus riparius*. Our work demonstrates that exposure to low and relevant levels of ALAN increases the sensitivity of *C. riparius* to other urban stressors. Light pollution decreases heat tolerance (lower Critical Thermal Maximum, CT_{max}) and reduces lethal tolerance of larvae to chemical stressors (glyphosate and salinity). Interactive effects, including synergisms, were also observed under combined exposures to ALAN and these stressors for the development and emergence (Mean Emergence Time, EmT₅₀) of *C. riparius*, suggesting that light pollution can have detrimental impacts on aquatic insects living in urban aquatic ecosystems.

The data obtained in this work that used low ALAN levels and realistic exposure scenarios for urban areas show that light pollution should be an important consideration in ecological risk assessment of chemical stressors including plant protection products. Results can also contribute to future legislation on light pollution and to a more effective protection of biodiversity and the ecological integrity of urban ecosystems.

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2.06.P-Th020 Effects and Fate of the Nitrification Inhibitor N-[3(5)-Methyl-1H-pyrazol-1-yl)methyl]acetamide and the Urease Inhibitor N-(2-Nitrophenyl)phosphoric Triamide in a Preliminary Freshwater Mesocosm Study

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Nitrification and urease inhibitors (NUIs) are applied as fertilizer additives in agriculture to reduce emissions of the greenhouse gases ammonia and nitrous oxide, as well as leaching of nitrate into groundwater. Their modes of action rely on the suppression of soil enzymes and bacteria that play a crucial role in the nitrogen cycle. Among the inhibitors approved in the EU, only Nitrapyrin was considered as hazardous to the environment during the approval process. Other NUIs, such as N-[3(5)-methyl-1H-pyrazol-1-yl)methyl]acetamide (MPA), 3-methylpyrazole (3-MP), and N-(2-nitro-phenyl)phosphoric triamide (2 NPT), are classified as carcinogenic, mutagenic, and reproductive toxic, respectively. 3-MP, which is both an active substance and a metabolite of MPA, is also under assessment for endocrine-disrupting properties. Previous research has primarily focused on terrestrial systems, whereas potential environmental risks in aquatic systems due to runoff events remain insufficiently studied.

Here, the fate of MPA and 2-NPT as well as their potential effects on aquatic organisms were investigated over 105 days using small pond mesocosms (2 m³) with sediment from June to September 2025. In an establishing period prior to NUI application, the mesocosms were inoculated with water, plankton, and periphyton from natural ponds. Subsequently, the mesocosms were spiked individually with 50 µg/L MPA and 2-NPT or as a mixture at 5 µg/L. For fate investigations, water samples were analyzed via LC-MS/MS at defined time points. At the end of the study, MPA was no longer detectable (< 0.005 µg/L) in the water phase, whereas 3-MP was still present at up to 65 % of the initial amount of MPA. 0.10 and 0.37 µg/L 2-NPT was detected after 105 days,

whereas the metabolite 2-NA disappeared after its formation during the course of the study. Nutrient analyses revealed changes in the dissolved nitrogen forms nitrate, nitrite, and ammonium depending on the applied NUI. Ex situ cultivation assays of bacteria showed, based on preliminary data, generally a declining trend of overall bacterial abundance and the abundance of different bacterial species over time. NUIs had distinct effects on phytoplankton, zooplankton, and periphyton. In-situ assays of duckweed and snails disclosed minor effects on growth and no effects on reproduction, respectively. Our study provides first insights into the fate of selected NUIs in freshwater ponds, as well as their differential effects on the organisms.

2.06.P-Th021 Transgenerational impacts of TBBPA on the freshwater snail *Lymnaea stagnalis*
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TBBPA, a brominated flame retardant (BFR), is a widely used plastic additive that poses ecological risks due its endocrine-disrupting potential, bioaccumulation and toxicity in aquatic environments. While its acute toxicity has been documented in several aquatic species, far less is known about the long-term and transgenerational effects in the freshwater snail *L. stagnalis*, limiting our understanding on long-term ecological consequences and its potential implication for population dynamics. Freshwater snails are key players in the ecosystem that helps in nutrient cycling and ecosystem stability. In this study, we assessed whether parental exposure to TBBPA will impact the vitality and early development of an unexposed F1 generation of exposed *L. stagnalis*, thereby addressing the carry-over effects.

Adult snails (F0) were exposed for up to 28 days to nominal TBBPA concentrations between 75 and 1500 µg/L. Egg masses produced by exposed parents (F1 embryos) on day 14 and 28 were transferred to 24-well glass plates containing clean medium to isolate carry-over effects from direct exposure. Additionally, embryos from unexposed parents were placed in a 24-well glass plate and exposed to nominal TBBPA concentrations of 50–1000 µg/L. Development of embryos was monitored for 14 days.

Parental exposure resulted in impaired reproduction, with reduced number of clutches and embryo/ clutch production as early as day 3 of adult exposure. Their embryos (F1) showed different types of abnormalities, including small shell, delayed hatching, low pigmentation and other malformations, even at the lowest nominal concentration. The EC₅₀ value was ~350 µg/L, suggesting transgenerational effects even without direct exposure. The F1-embryos that were further monitored for chronic effects didn't not compensate their abnormalities (e.g. smaller shell size) by the end of the observation period of 3 month. Embryos of the non-exposed parents showed similar abnormalities with an EC₅₀ of 235 µg/L after 14 days and 100% mortality at 1000 µg/L.

This finding reveals that TBBPA can impair offspring vitality even in the absence of continued exposure, suggesting potential long-term consequences for population stability. These findings highlight the need to consider both exposure pathways and transgenerational effects when assessing the ecological risk of high-Kow, bioaccumulative and hydrophobic contaminants.

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2.06.P-Th022 A Risk Evaluation of the Ubiquitous Ultraviolet (UV)-Filter Octocrylene
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Octocrylene is an ultraviolet (UV)-B filter and sunscreen stabilizer that is used in sunscreens and personal care products and is the most frequently used UV filter in cosmetics. Sources in the environment are diverse and include sunscreen, machine washing liquids/detergents, automotive care products, paints, coatings or adhesives and fragrances, as well as construction materials, furniture, and flooring which enter aquatic ecosystems through runoff and wastewater effluents. Subsequently, recent reporting shows it is commonly found in recreational freshwater (geometric mean: 30 ng/L, maximum: 4.66 µg/L) and marine (geometric mean: 37 ng/L, maximum: 10.6 µg/L) ecosystems, as well as in sediment, but its concentrations vary considerably temporally and spatially.

It has been identified as hazardous to the aquatic environment through long-term chronic studies and has been determined by ANSES to be a persistent and toxic compound but not bioaccumulative. Since octocrylene has low solubility in water, and a high log K_{ow} (6.1), its effects on sediment-dwelling organisms are also potentially important. Ecotoxicity studies have been done for several trophic levels of freshwater and marine species, particularly in literature studies, and the risks to marine organisms is a growing area of research interest. Additional studies on the endocrine activity of octocrylene and its major metabolite, benzophenone, have been conducted as there are indications of endocrine activity from both compounds. Due to its widespread detection in the environment and its potential for aquatic toxicity, an assessment on the risk to aquatic and sediment-dwelling organisms was undertaken. Using the existing regulatory submission study reports and literature search results, environmental quality standards for octocrylene in surface waters and sediments were derived. Literature studies were assessed using the CRED approach, which takes into account the reliability and relevance of the study even when non-standard species or non-guideline studies are done. While no mesocosm data was available and there were not enough datapoints for a species sensitivity distribution (SSD) derivation, there was enough relevant and reliable data for the derivation of EQS values for acute and chronic aquatic ecosystems and sediment ecosystems, and to assess the risk for secondary poisoning to higher tier organisms.

2.06.P-Th023 Synergistic Threats: How Temperature and 6PPD-Q Contamination Reshape Arctic Char Development in Alpine Lakes

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Freshwater biodiversity faces severe threats from anthropogenic stressors, notably climate warming and emerging contaminants such as 6PPD-quinone (6PPD-Q), a highly toxic byproduct of tire wear. While 6PPD-Q is recognized for its acute lethality in species like coho salmon (*Oncorhynchus kisutch*), its synergistic effects with rising temperatures on cold-water fish—particularly during critical early life stages—remain poorly understood. Arctic charr (*Salvelinus umbla*), a cold-stenothermal species endemic to alpine lakes, may be especially vulnerable due to its limited thermal tolerance and increasing exposure to urban runoff.

In this study, we investigated the combined impacts of temperature (5°C and 8.5°C) and 6PPD-Q contamination (0, 1.0 and 3.5 µg/L) on larvae from two Arctic charr populations: one native and cold-adapted (Lake Geneva) and one introduced and putatively more thermally tolerant (Lake Annecy).

We assessed a range of traits to elucidate potential synergistic effects on larval development: Physiological (survival, metabolism, and oxidative stress via GST and catalase activity); Morphological (malformation and growth rates); Behavioral (swimming behavior).

Our results revealed significant interactive effects between temperature and 6PPD-Q, demonstrating that warming exacerbated the toxic effects of the contaminant. The chorion provided partial protection during embryogenesis, but post-hatch larvae were highly sensitive to the combined stressors. Annecy charr broodstock are raised in aquaculture for 1 to 5 years in water with a relatively stable temperature of 7 to 10°C.

This acquired thermal tolerance is likely transmitted to their offspring, suggesting a form of transgenerational effect or epigenetic inheritance of thermal stress adaptation. Contamination induced significant behavioral alterations (e.g., reduced swimming activity) and elevated oxidative stress, likely linked to underlying metabolic and cardio-respiratory disruptions.

These findings underscore the significant ecological risks posed by combined thermal and chemical stressors and highlight the crucial role of population-specific adaptations in environmental risk assessments. Our work calls for integrated conservation strategies that explicitly account for local genetic backgrounds and synergistic stressor interactions to effectively protect vulnerable cold-water species in a rapidly changing climate.

2.06.P-Th024 Synthesis, characterization and acute toxicity evaluation of functionalized maghemite nanoparticles in the ostracod *Cypridopsis vidua*.

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The increasing use of nanomaterials in industry, biotechnology, medicine and human consumption requires a toxicological assessment of their impact on aquatic ecosystems. In this study, maghemite (γ-Fe₂O₃) nanoparticles were synthesized by coprecipitation and functionalized with sodium citrate dihydrate.

Subsequently, they were characterized by X-ray diffraction analysis to determine their elemental composition and particle size, and then the acute toxicity of the maghemite nanoparticles was evaluated in juvenile ostracods of *Cypridopsis vidua*. The ostracods used were isolated from a karst aquatic ecosystem of the Yucatán Peninsula

and cultured monoclonal under laboratory conditions at 25°C. A 48-hour exposure bioassay was performed in sterile 24-well polystyrene plates, exposing 100 organisms per treatment to concentrations of 20, 50, 100, 200, 400, 500, 600, 800, and 1000 mg/L (5 organisms per well in 2 mL of nanoparticle solution). The dose-response relationship was analyzed using a log-logistic model with R software. The results showed an upward-sigmoid trend; however, mortality did not exceed 20% even at the highest assay concentration (1000 mg/L). Due to this low toxicity, the Median Lethal Concentration (LC50) values were estimated by extrapolation, resulting in an LC50 > 1000 mg/L. These findings suggest that the synthesized functionalized maghemite nanoparticles have low acute toxicity for ostracods, as the concentration required to affect 50% of the population significantly exceeds standard experimental limits and the conditions expected in natural environments. The demonstrated low toxicity of maghemite nanoparticles to aquatic organisms like *Cypridopsis vidua* is a finding of paramount importance. Being a biocompatible and

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2.06.P-Th025 The eyes are the window to the sea: Metals and Metalloids in the ocular tissues of a coastal shark from Southeastern Brazil

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Elasmobranchs are among the most threatened vertebrates worldwide, yet they remain critically understudied in terms of ecotoxicological impacts. This group relies heavily on vision for navigation, foraging, and predator avoidance, yet almost nothing is known about how metals accumulate within their ocular tissues, an increasing issue in coastal environments. In this context, this study aimed, for the first time, to assess different accumulation profiles in the different eye structures (cornea, lens, sclera, and choroid) metal in *Rhizoprionodon lalandii*, a small coastal shark in southeastern Brazil. Individuals were sampled from artisanal fisheries, eyes were dissected into distinct tissues, and elemental concentrations were quantified by ICP-MS, and age classes (adults, juveniles, and neonates) and sampling locations were compared. A consistent distribution pattern emerged across groups, where the cornea was the primary accumulation site for most elements, including Fe, Mn, Sr, Ti, Zn and, in some cases, Co and Ni, while the lens generally exhibited the lowest concentrations. The sclera often paralleled the cornea with high Mn, Sr, Ti, and Zn levels, suggesting similar affinities for these elements. The choroid displayed more variable behavior, accumulating certain elements such as Se in adults and As in neonates. Despite these variations, some elements showed little tissue differentiation, while others, such as Rb, Hg, or As, displayed age-specific or structure-specific preferences. Overall, the observed metal distribution within shark eyes seems to be highly structured, with the cornea and sclera consistently acting as key accumulation tissues for multiple elements, the lens serving as a low-accumulation compartment, and the choroid reflecting more specialized or dynamic patterns. These findings advance our understanding of ocular physiology under environmental contamination and provide a basis for evaluating how metal exposure might affect sensory performance in coastal shark species, guiding future ecotoxicological assessments and conservation efforts for elasmobranchs living in polluted habitats.

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2.07 Identifying Safe Operating Spaces for Biodiversity in Water and Sediment Systems

2.07.T-01 Trends and Thresholds of River Macroinvertebrate Biodiversity Responses to Anthropogenic Stress for Identifying Safe Operating Spaces

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Freshwater ecosystems globally face multiple environmental pressures, including climate and land-use change, altered hydrology and chemical pollution. These pressures threaten biodiversity and challenge efforts to balance ecological protection with societal needs. This study evaluates how riverine macroinvertebrate biodiversity responds to chemical pollutants within a multi-stressor context, with the goals of identifying consistently responding biodiversity metrics and determining levels of toxic pressure at which ecological degradation accelerates.

To this end, European macroinvertebrate time-series data from river sites distributed across 23 countries, spanning multiple decades was analysed. Multiple community, taxonomic, trait-based biodiversity metrics were derived. Environmental parameters included land cover, climate indices and modelled toxic pressure. Variable selection was performed using elastic-net regularisation with cross-validation, and selected biodiversity–stressor relationships were quantified using generalized additive models with spatial and temporal structure and site-level random effects. Change-points were explored using modelled response curves.

Most taxonomic and trait-based indicators demonstrated clear declines with increasing toxic pressure, even after accounting for climate and land-use covariates. Sensitive groups, such as insect orders that have low tolerance to pollution or taxa characterised by low dispersal capacity, showed particularly strong negative responses. Several metrics displayed non-linear patterns, with steep declines beginning at low levels of toxic pressure. In contrast, community- and functional metrics generally exhibited weaker or inconsistent responses, suggesting greater ecological redundancy or slower turnover. Although distinct thresholds were not sharply defined, the results indicate that adverse biodiversity responses likely emerge at low levels of chemical impact, warranting further investigation of low impacted sites.

Overall, this work demonstrates that chemical pollution exerts a strong, independent constraint on aquatic biodiversity across Europe, compounding the influence of climate and land-use stressors. These findings highlight the need for integrated strategies that simultaneously address chemical, climatic and habitat pressures to maintain ecological integrity and define safe operating spaces for freshwater ecosystems.

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2.07.T-02 Real-time, high-throughput biodiversity monitoring reveals impacts of chemical mixtures in freshwater ecosystems

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Understanding how chemical mixtures, compounded by other stressors, impact freshwater biodiversity across space and time is essential for protecting ecosystems. We developed a scalable, high-throughput framework that integrates biodiversity quantified through environmental DNA, untargeted chemical analysis, and multimodal machine learning to identify biodiversity shifts and their drivers in freshwater ecosystems. Applying this approach to 52 English lakes, we found that spatial biodiversity patterns were strongly shaped by plant protection products and metals, with insecticides and fungicides emerging as key contributors to biodiversity loss. Lake typology and land use also played significant roles.

To explore temporal dynamics, we applied the same framework to a 100-year sediment archive from a lake with a well-documented history of human impact. Despite improvements in water quality, community composition and ecosystem functions exhibited irreversible changes. Biocide mixtures and climate extremes together explained up to 90% of observed biodiversity shifts over time. These findings expose critical limitations in traditional monitoring approaches and demonstrate the value of holistic, data-driven methods to identify and prioritize threats to biodiversity.

To support real-world uptake, we developed an in-field DNA monitoring platform combining our multiplex

metabarcoding protocol, which enables screening of entire freshwater communities in a single reaction, with portable Oxford Nanopore sequencing. This system provides rapid, low-cost, real-time biodiversity data, empowering stakeholders to monitor ecological status and act quickly in response to emerging risks. Looking forward, we are expanding our temporal analysis to national scales by reconstructing pollution and biodiversity trajectories from lake sediment archives. By linking persistent and bioaccumulative chemical mixtures with historical biodiversity, ecosystem functions, climate, and land use data, we aim to identify and rank threats to biodiversity using AI-enabled analytics. Long-term records are pivotal for forecasting biodiversity loss under future scenarios, enabling regulators to intervene early and preserve critical freshwater resources.

Our integrated approach offers a forward-looking, scalable solution to monitor, understand, and mitigate the ecological impacts of chemical mixtures, advancing sustainable freshwater management and aligning with EU biodiversity and Net Zero goals.

2.07.T-03 The freshwater amphipod *Gammarus fossarum* as European model species for substance toxicity assessment using spiked-sediment

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A new OECD test guideline (TG) on freshwater amphipods is currently under development (“Sediment-Water Amphipod Toxicity Test Using Spiked Sediment”, Project 2.68) and validation through interlaboratory assay. The protocol focusing on the North-American amphipod *Hyaella azteca* assesses toxicity of persistent chemicals in sediment and comprises a chronic toxicity test that measure the effect on adults’ reproduction. Here we aim at highlighting the interest for including in this new TG the European freshwater amphipod *Gammarus fossarum*. To do so, we first determined if the composition and the aging duration of five formulated sediment included the one used in OECD protocol affected gammarids’ life-history traits (i.e. survival and fecundity). We found no effect of sediment aging on females’ survival after 18 days but we highlighted a significant effect of composition, sand alone being the worst sediment. No significant effect of sediment composition, aging or interaction was found on the number of embryos per females as reproduction endpoint. In 2025, we initiated an interlaboratory assay with several European countries to determine the effect of two molecules (bifenthrin and pyrene) selected for *H. azteca* validation test on gammarids’ reproduction (i.e. juveniles production). The proposed protocol is a 29-day chronic toxicity test during which adults couples are exposed to sediment spiked with different concentrations of bifenthrin or pyrene for 18 days, then females with developing embryos are transferred in control media so that they can release juveniles during an observation period of 11 days. Regarding our first results on pyrene exposure, females’ survival in the control condition met the validity criteria established for the test (? 60%). No significant concentration-dependent effect of pyrene was observed on survival but we found a significant decrease in number of juveniles produced per females after female exposure to pyrene-spiked sediment at the highest concentration (640 mg/kg). Overall, we demonstrated that *G. fossarum* is a suitable alternative species for the evaluation of persistent chemicals through sediment-water toxicity test.

Key-words: ecotoxicology; laboratory exposure; reproduction; OECD; gammarids

2.07.T-04 Reflecting on 20 Years of OECD 225: Advancing Standardised Testing With *Lumbriculus Variegatus* With Ecotoxicological Insight

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Sediments are key environmental compartments that both accumulate and release chemical contaminants, leading to chronic exposure of benthic communities. The freshwater oligochaete *Lumbriculus variegatus* is the only sediment-dwelling model species recommended for sediment risk assessment under OECD guidelines. Despite its ecological relevance and high sediment ingestion rate, *L. variegatus* is used considerably less often than *Chironomus* sp. in ecotoxicological sediment studies.

Here, we systematically reviewed 302 ecotoxicological studies on *L. variegatus* published between 2007 and 2025, identifying 91 studies that met predefined inclusion criteria (e.g., laboratory-based chemical exposures,

assessment of ecotoxicological effects) and methodological quality standards (chemical analysis, use of controls, number of replicates). Only 10% of the chronic sediment studies (?28 days) strictly followed OECD Guideline 225 without deviations (e.g., additional food supplement, lack of synchronisation), whereas 40% did not adhere to the guideline at all. Conventional endpoints (mortality, growth, reproduction) were measured in 92% of all studies; however, life-stage differentiation was rarely applied, and 24% of the chronic studies failed to meet the reproduction criterion.

In total, 41% of the studies extended OECD 225 by measuring non-conventional endpoints (i.e., endpoints not described by the guideline). Behavioural measures (feeding, locomotion, sediment avoidance, and bioturbation) were most frequently used (29%), and often proved more sensitive than conventional endpoints. Comparative studies with *Chironomus* sp. (26 studies) revealed no consistent pattern of relative sensitivity, with observed differences largely attributed to exposure route, feeding behaviour, species-specific biotransformation capacities, and endpoint selection.

Our review identifies major gaps in standardisation, endpoint selection, and experimental design for *L. variegatus* studies. We recommend revising OECD 225 to include non-conventional endpoints, redefining synchronisation periods prior to exposure, synchronising organisms 14 days prior to exposure, and differentiating life stages for growth and reproduction. Implementing these improvements will enhance the ecological relevance and regulatory applicability of *L. variegatus* in sediment ecotoxicology. This work synthesises nearly two decades of research and provides a clear roadmap for advancing sediment risk assessment.

Disclaimer/Disclosure:

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2.07.T-05 Multigenerational Disruption of Reproduction and Behaviour in a Benthic Worm Exposed to Sediment-Associated Antidepressants.

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Pharmaceuticals are increasingly recognized as emerging environmental contaminants, with selective serotonin reuptake inhibitors (SSRIs) such as sertraline of particular concern due to their widespread use and persistence in aquatic systems. Owing to its lipophilic, ionizable properties, sertraline accumulates in sediments and may become bioavailable to benthic deposit feeders like *Capitella teleta*, a species central to nutrient cycling and food web dynamics. To address knowledge gaps on long-term impacts, we investigated the effects of environmentally relevant sediment-associated sertraline concentrations across three generations of *C. teleta*. Both conventional life-history endpoints and behavioural responses were assessed to capture subtle, multigenerational effects. Sediment-associated sertraline caused generation-dependent changes in reproduction and behaviour of *C. teleta*, revealing trade-offs between reproductive effort, offspring quality, and locomotor function. These findings stress the need for multigenerational approaches and behavioural endpoints to better capture long-term ecological risks of pharmaceuticals in benthic systems. Results highlight the importance of extended exposure designs for evaluating sublethal pharmaceutical impacts on benthic invertebrates and their ecological functions.

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2.07.P Identifying Safe Operating Spaces for Biodiversity in Water and Sediment Systems

2.07.P-Th026 Assessing Toxicity of Emerging Semiconductor Pollutants Gallium and Indium in Contaminated Paddy Soils with A Soil–Water–Fish System

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Gallium (Ga) and indium (In) are critical metals widely used in semiconductor and optoelectronic industries, where their compounds are indispensable to modern electronic devices. However, improper disposal and industrial effluents have led to their emergence as environmental contaminants, particularly in agricultural systems such as paddy fields. The environmental fate, ecological toxicity, and potential risks of these types of pollutants in the agroecosystem remain poorly understood. In this study, a static soil–water–fish exposure system was developed using juvenile rice fish, medaka (*Oryzias latipes*), as a model organism to investigate the bioavailability and toxic effects of Ga and In in two contrasting paddy soils: acidic, aluminum (Al)-rich Pingzhen (Pc) soil and neutral, low-Al Sangkang (Su) soil. Sequential and KCl extractions revealed that exchangeable Al in Pc soil increased markedly with rising Ga or In concentrations. Soil pH and Al content strongly influenced metal mobility and bioavailability—Su soil released more soluble Ga, while Pc soil released more soluble In, with Ga notably enhancing Al mobilization from soil to water phases. Medaka exposed to highly contaminated Pc soils (>1 mmol/kg Ga or >4 mmol/kg In) exhibited significant mortality, likely due to Al displacement and release. In Su soils, no significant mortality was observed, but exposed fish accumulated Ga or In in a dose-dependent manner, resulting in sublethal effects such as growth inhibition and altered swimming behavior even at lower exposure levels. The soil–water–fish exposure system established provides an effective method for assessing the agroecological risks of emerging semiconductor-related metals for environmental monitoring and soil quality guidelines to ensure sustainable rice production and food safety.

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2.07.P-Th027 Formulated sediment: a good control for bioassays to assess freshwater sediment toxicity on *Gammarus fossarum*?

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Freshwater sediments act as sinks for pollutants like metals, polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), pharmaceuticals and personal care products (PPCPs), per- and polyfluoroalkyl substances (PFAS). While chemical analyses are the primary tool for studying sediment contamination, it is now useful to complete them with bioassays to provide effect-based sediment quality assessment¹. In order to perform those tests, standardized guidelines recommend the use of a formulated sediment as a laboratory control (eg. OECD 218). This formulated sediment is mainly composed of quartz sand, kaolin and peat and can be used for soil organisms (nematodes, ISO 10872), aquatic plants (watermilfoils, OECD 239), and benthic macroinvertebrates (amphipods, NF T 90-338-1).

In an effort to develop a new bioassay with a European amphipod, *Gammarus fossarum*, the relevance of the control sediment is questionable.

Two biomarkers, feeding rate and fertility, have been studied on gammarids exposed to 16 formulated sediments (with 3 replicates for each test) and 127 natural sediments (with 5 replicates for each condition) in order to assess their toxicity. Could this formulated sediment be good control to discriminate natural sediments depending on their toxicity?

First, the reproducibility of the formulated sediment was studied. Then, it was compared to uncontaminated natural sediments to see if the same amount of sediment is considered as toxic depending on the control used. While formulated sediment seems to be a good laboratory control for the reproduction test. It is not as efficient for the feeding rate test. Indeed, it does not allow the detection of as much toxic sediments as when using natural sediments considered to be uncontaminated.

Therefore, we propose to establish reference values, based on statistical method already used for those biomarkers^{4,5}, using the large dataset we have. At the end of this oral presentation, everybody will be able to do two bioassays to assess sediment quality using *Gammarus fossarum* and to interpret the results.

This work is aligned with the session 2.02 «Buried Risks and Hidden Insights: Advancing Sediment Quality Assessment for Ecosystem and Human Health».

2.07.P-Th028 Species Chemical Sensitivity Comparison Between Freshwater Amphipod *Hyaella azteca* and *Gammarus* spp.

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Toxicity tests with the North American amphipod *Hyaella azteca* are widely used for regulatory chemical risk assessment. However, *H. azteca* is not present in some regions, where native *Gammarus* spp. are more appropriate test organisms. To evaluate whether *Gammarus* spp. could serve as alternative species in a chronic sediment–water amphipod test method (that is under development at OECD as new test guideline (project 2.68)), we compared their sensitivity with that of *H. azteca* using the USEPA ECOTOX knowledgebase. We extracted 96-h LC50 values for water column exposure tests, because data on spiked sediment toxicity were limited for a robust comparison. Then we excluded censored values (< or >), and removed records exceeding 10 times aqueous solubility. When multiple LC50 values existed for the same chemical–species combination, we calculated geometric means. The final dataset comprised 780 LC50 records for 240 chemicals, including 203 records for *H. azteca* (64 chemicals) and 577 records for six *Gammarus* species (*G. fasciatus*, *G. fossarum*, *G. italicus*, *G. lacustris*, *G. pseudolimnaeus*, and *G. pulex*). For most chemicals (64%–90% chemicals that had data available for both taxa), the LC50 values for *H. azteca* and *Gammarus* spp. differed by less than 10-fold, indicating no clear interspecies differences. In addition to direct LC50 comparisons, we characterized the distribution of log-transformed LC50 values and examined the fifth percentile for all chemicals. As a result of 1,000 times bootstrap iterations, the 95% confidence intervals of fifth percentile values for *H. azteca* and *Gammarus* spp. strongly overlapped. Fifth percentile LC50 estimates and their confidence intervals were similar for both amphipod groups, even when chemicals were limited to specific classes (e.g., narcotics, acetylcholinesterase inhibitors). These results indicate that, on average across a broad range of organic chemicals, the acute toxicity towards *Gammarus* spp. is neither consistently more nor less than that of *H. azteca*. Our analysis supports the use of *Gammarus* spp. as appropriate alternative species for implementing an OECD chronic sediment–water amphipod test.

2.07.P-Th029 Interlaboratory Validation of Chronic Sediment–Water Amphipod Toxicity Test with *Hyaella Azteca*

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Chronic sediment–water toxicity tests with benthic organisms are crucial for the risk assessment of hydrophobic and sediment-associated chemicals. However, internationally harmonized long-term methods are still limited. Within an OECD-approved project, we conducted interlaboratory validation tests of a 42-day sediment–water toxicity test using the freshwater amphipod *Hyaella azteca* and spiked formulated sediment. The amphipods were exposed to the spiked sediment for 28 days and then transferred to clean water for 14 days to observe reproduction. A total of seven laboratories from Europe, North America, and Japan participated; five conducted tests with the pyrethroid insecticide bifenthrin and six with the polycyclic aromatic hydrocarbon pyrene. At all laboratories, the concentrations of test chemicals in sediment were measured.

For bifenthrin, all five laboratories met the predefined validity criteria (control survival ? 80%, dry weight per surviving amphipod ? 0.50 mg, number of young amphipods per adult female ? 6.0, and basic water quality parameters), whereas four of six laboratories met the criteria for pyrene. Both test chemicals remained mostly in sediment phase throughout exposure period at all laboratories. Across laboratories, differences in NOEC values for survival, biomass, and reproduction were within a factor of four for bifenthrin, but those for pyrene exceeded a factor of 10 for pyrene due to the outlier values from laboratories that did not meet the criteria. When data from these two laboratories were excluded, coefficients of variation (CVs) for NOEC and EC50 values based on measured sediment concentrations fell between 31% and 94%, comparable to a previous interlaboratory chronic validation test with *H. azteca* using field-contaminated sediments.

These results demonstrate that the proposed 42-day sediment–water test with *H. azteca* yields reproducible chronic toxicity evaluation across a broad range of laboratories and test designs. The validated protocol provides a robust basis for regulatory sediment risk assessment.

2.07.P-Th030 Sedimentary Co-exposure of Polystyrene Nanoplastics and Lithium Bis(trifluoromethanesulfonyl)imide Synergistically Exacerbates Ecotoxicity and Risk in *Caenorhabditis*

elegans

Bo-Wei Jhang and Vivian Hsiu-Chuan Liao, National Taiwan University, Taiwan (Greater China)

The increasing production of lithium-ion batteries and the pervasive presence of nanoplastics have raised concerns about their coexistence and combined impacts in sedimentary ecosystems. Yet, the interactive ecotoxicological effects of these emerging contaminants remain poorly understood. This study aimed to assess the joint toxicity of lithium bis(trifluoromethanesulfonyl)imide (LiTFSI), a lithium salt widely used in battery electrolytes, and polystyrene nanoplastics (PS-NPs) using *Caenorhabditis elegans* as a sediment-dwelling model organism. Worms were exposed for 96 hours to various concentrations of LiTFSI and environmentally relevant PS-NPs (0.015 µg/g), alone and in combination. Growth, reproduction, and molecular endpoints were evaluated, and toxicity interactions were analyzed using Hill modeling, Bliss independence analysis, and DEBtox modeling. Results showed that PS-NPs significantly amplified LiTFSI-induced developmental and reproductive toxicity. Bliss analysis confirmed a synergistic effect between the two contaminants, while DEBtox modeling indicated that PS-NPs shifted LiTFSI's physiological mode of action from reproduction impairment to increased maintenance costs. At the cellular level, PS-NPs enhanced LiTFSI-induced mitochondrial stress, fragmentation, and germline DNA damage. Risk quotient assessments based on sediment data from Belgium, the United States, and China further suggested that co-exposure of PS-NPs and LiTFSI substantially elevates ecological risks. In conclusion, this study demonstrates that environmentally relevant level of nanoplastics can exacerbate the toxicity and ecological risk of lithium battery-related contaminants, emphasizing the need to consider co-contaminant interactions in environmental risk assessment and sediment management.

Disclaimer/Disclosure:

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2.07.P-Th031 The Effect of Elevated Temperature on Pesticide Terbutylazine and Antimicrobial Triclosan Toxicity to *Daphnia magna*

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Anthropogenic chemical pollution poses one of the largest environmental threats to humanity, yet global understanding of the issue remains fragmented. Water pollution is alarming because anthropogenic activities contaminate water bodies with various pollutants, which in turn reduce ecosystem functioning and pose human health risks. Pesticides and pharmaceutical and personal care products (PPCPs) stand out for their widespread occurrence and highly diverse biological effects and are considered emerging contaminants (ECs). In addition to the direct stress caused by chemical contamination, there are certain environmental stressors (such as elevated temperature) that might cause additional stress. Thus, it is important to understand the effects of changing environmental factors on pollutants' ecotoxicity to aquatic organisms. To address the knowledge gap of the effects of emerging contaminants in a changing climate, we aimed to test the ecotoxicological effects of chronic exposures to the frequently detected pesticide Terbutylazine (TBA) and antimicrobial Triclosan (TCS) on *Daphnia magna*. *D. magna* was exposed to 5 different single TBA and TCS concentrations under different temperature regimes (22°C and 26°C) for 21 days. Mortality and reproduction rates as well as body size of *D. magna* were observed.

2.07.P-Th032 An Integrated Weight-of-Evidence Approach for Establishing Causality between Heavy Metal Contamination and Ecological Impacts in Aquatic Ecosystems

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Establishing a robust causal link between heavy metal contamination and observed ecological impairments remains a critical challenge in environmental risk assessment. This study presents an integrated Weight-of-Evidence (WoE) framework that combines the empirical Sediment Quality Triad (SQT) approach with John Norton's philosophical causal justification framework to assess the ecological impact of metals in aquatic ecosystems. The assessment integrates three distinct lines of evidence (LOE): (1) chemical assessment using Hazard Quotients (HQ) for surface water and mean Probable Effect Level quotients (mPELq) for sediments; (2) toxicological evaluation through sediment and elutriate toxicity tests; and (3) biological assessment utilizing a stressor-specific ecological index (SPEAR_metal) alongside traditional community metrics. Applied to a case

study in the Nakdong River basin, South Korea, the integrated framework successfully linked exposure to ecological responses. The chemical LOE identified spatial hotspots of contamination, while the toxicological LOE confirmed adverse effects consistent with metal concentrations. Crucially, the biological LOE demonstrated that the SPEAR_metal index exhibited a stronger and more significant negative correlation with metal gradients compared to general diversity indices, effectively serving as evidence for "specific alteration." Through hierarchical integration of these evidences, Zinc (Zn) and Cadmium (Cd) were identified as the primary causative agents driving ecological degradation among the complex mixture of pollutants. This study validates the utility of SPEAR_metal as a key diagnostic tool and demonstrates that the proposed causal confidence-based WoE framework significantly enhances the transparency and defensibility of ecological assessments in complex aquatic environments.

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2.07.P-Th033 Life of a sediment dweller in a mesocosm – Strategies to assess sediment communities in regulatory mesocosm studies for PPP risk assessment

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Sediment-dwelling organisms play a critical role in the ecological functioning of waterbodies in agricultural areas and form part of the environmental risk assessment of plant protection products (PPPs), albeit only one representative species is included as a standard test species (Chironomids). While laboratory tests provide controlled conditions for evaluating chemical effects, they often lack the environmental realism needed to capture community-level responses. Mesocosm systems bridge this gap by integrating multiple biological compartments and their interactions, temporal dynamics, and environmentally relevant exposure pathways. However, the assessment of sediment organisms within mesocosm studies remains methodologically challenging due to the heterogeneous sediment structure, low organism densities, and practical constraints associated with sampling without disturbing the system's integrity.

At CEA, we utilise flat-bottomed aquatic mesocosm test systems, designed to simulate natural freshwater communities, including benthic invertebrate assemblages. Recently, we have been investigating ways to improve sampling strategies and analytical approaches for sediment-associated endpoints, with a particular focus on enhancing robustness and statistical power, as well as reducing intrinsic variability. Our method development work includes optimisation of sediment coring techniques, refinement of subsampling procedures, evaluation of organism recovery efficiency, and an assessment of spatial variability across replicates. We have also explored how sampling frequency and timing influence the detectability of PPP-related effects on benthic communities.

This poster presents our methodological advancements to date, compares multiple sampling approaches tested within our mesocosm system, and discusses implications for generating robust, ecologically relevant data to support regulatory ecotoxicology risk assessments. Our results are intended to contribute to ongoing efforts within the mesocosm research community to improve data quality and strengthen the reliability of sediment organism endpoints in higher-tier PPP assessments.

2.07.P-Th034 Sediment-Water Toxicity Testing Beyond Standard Guidelines: Insights from Using Non-Guideline Aquatic Invertebrates

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Invertebrate toxicity testing is an important component in the registration process for today's modern chemicals. Although current tier 1 testing requirements primarily focus on standard laboratory-cultured aquatic invertebrates, there is an increasing interest in expanding the range of species evaluated to enhance environmental risk assessments. In recent years, the demand for testing a broader range of invertebrate species for higher tier risk assessment studies has steadily increased to support the development of species sensitivity distributions (SSD) for European risk assessments and for Endangered Species evaluations in the US. Current

OECD and OCSPP guidelines for invertebrate testing include daphnids, mysids, amphipods, oligochaetes and chironomids. To develop species sensitivity distribution, non-guideline aquatic insects such as the stonefly, caddisfly and mayfly were incorporated to provide additional toxicity data. SSD testing has commonly been utilized for acute testing in water only exposures based on OECD Guidelines 202 and 235. For chronic testing, SSD approaches have been implemented in sediment/water systems in accordance with OECD Guidelines 218 and 219. Sediment-water toxicity testing with non-standard and non-guideline freshwater aquatic invertebrates introduces unique challenges for Good Laboratory Practice (GLP) studies. In this work, we summarize our experience conducting GLP-compliant acute and chronic toxicity tests with additional aquatic invertebrate species and discuss critical factors influencing study success, including test design, organism availability and age, acclimation procedures, and dietary requirements. Key takeaways and practical considerations are presented to inform future testing strategies and support method development for expanding species coverage beyond standard guidelines.

2.07.P-Th035 Toxicity evaluations in coastal sediments using fish embryos and explorations of causative contaminants

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Chemicals released through human activities are transported to coastal areas by rivers, rainfall, and other pathways, and ultimately settle in sediments. Some pollutants are poorly degradable and can remain buried for long periods due to ongoing sedimentation, potentially affecting benthic organisms individually or in combination. To assess these impacts, we have developed a method that uses fish embryos to evaluate field-collected sediment toxicity. Because embryos are placed directly on contaminated sediments with only a small amount of pore water and no overlying water, this approach isolates sediment-driven toxicities.

Using this method, earlier toxicity assessments revealed areas of severe biological impact risk in Osaka Bay near Osaka and Kobe, in Ise Bay near Nagoya (home to Japan's largest port), and in Hiuchi-nada of the Seto Inland Sea. To examine the persistence of these impacts, we resampled the same sites one to several years later and reassessed risks using embryos. Several sites continued to exhibit high biological impact risk, while at others the sediment-related biological effects were minimal or undetectable. Conversely, new biological impacts emerged at sites that had previously shown no sediment toxicity. Even in areas with consistently high risk, some sites displayed markedly different effect patterns in fish embryos, suggesting shifts in the causative agents over time.

We measured representative sediment pollutants, including polycyclic aromatic hydrocarbons (PAHs), but changes in PAH concentrations did not explain the observed shifts in biological impacts. These findings indicate that sediment-associated biological risk can fluctuate relatively rapidly and that the causative substances may change over time. Although sediments are generally thought to exhibit smaller temporal changes in chemical concentrations than the water column, chemical monitoring alone may be insufficient to infer biological impact risk.

To further pinpoint the causative agents, we prepared multiple extracts from collected sediments using solvents with differing physicochemical properties or column chromatography and inferred agent characteristics from the toxicity profiles of each extract. Comprehensive chemical analyses of compounds within each fraction are underway to aid identification of the responsible agents. An overview of these ongoing efforts will be presented.

2.08 Integrating Evolution and Mechanisms to Advance Ecotoxicology Under Global Change

2.08.T-01 Effects of Adaptation on Evolutionary Potential in Multi-stressor Environments: Insights from Sequential Laboratory Evolution Experiments.

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Populations are increasingly confronted with multiple, often novel, combinations of environmental stressors that threaten their persistence and adaptive potential. Using sequential evolution experiments with cyclic parthenogenetic rotifers, we examined how prior adaptation to heat stress shapes subsequent evolutionary responses to copper stress, and vice versa. Our results reveal asymmetric cross-tolerance: adaptation to heat increased tolerance to copper, whereas adaptation to copper provided no protection against heat stress. Furthermore, heat adaptation was weakened when it was either preceded or followed by multigenerational

exposure to copper. In contrast, copper adaptation remained robust regardless of whether populations experienced heat stress before or after selection. These findings highlight the complex ways in which stressors interact to influence microevolutionary trajectories. Understanding such dynamics is crucial for predicting population persistence in variable, multi-stressor environments and carries important implications for biodiversity management under climate change and anthropogenic pollution.

2.08.T-02 Epigenetic Plasticity Enables Copepods to Cope with Ocean Acidification

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Plasticity enhances species fitness and survival under climate change. Ocean acidification poses a potential threat to copepods, a major zooplankton group that serves as a key link between the lower and higher trophic levels in the marine environment, yet the mechanisms underlying different adaptive responses remain poorly understood. Here we show that although elevated CO₂ can exert negative effects on reproduction of *Paracyclops nana*, multigenerational plasticity can enable recovery after three generations. By integrating the methylome and transcriptome with the draft genome and undertaking DNA methylation treatments, we demonstrate the vital role of epigenetic modifications in ocean acidification responses and identify regions associated with reproductive resilience. Our results demonstrate that DNA methylation might play an important role in enhancing species fitness of copepods and that failing to consider phenotypic plasticity could lead to overestimation of species' vulnerabilities.

2.08.T-03 Springtails in Distress - How Are Soil Fauna Impacted by Pesticides in a Changing Climate?

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Climate change, pollution and biodiversity loss rank among the most pressing environmental challenges. The 4-year project MULTICLIM -Effects of climate change in a multiple stress multispecies perspective- studied how climate change and pollution combined may affect soil fauna on individual, population, species and community levels. Effects included were somatic growth, maturation, age-specific reproduction, mortality and species abundance. We focused on how temperature and humidity conditions may alter responses in springtails and other soil fauna to the neonicotinoid imidacloprid, which is widely used in global crop production. We compared springtail species and population sensitivity to climatic conditions and pesticides exposure in two species adapted to different habitats; surface dwelling *Hypogastrura viatica* and soil dwelling *Folsomia quadriocculata* (Collembola), in populations adapted to Temperate and Arctic climatic regions of southern Norway and Svalbard, by combining laboratory experiments and population modelling. Effects on soil fauna diversity following a single dose of imidacloprid under different watering regimes were studied in a 3-month field experiment.

Both springtail species were more sensitive to imidacloprid than commonly used model springtails, and pesticide toxicity increased with climatic stress conditions in a population-dependent way: Temperate populations were most sensitive to elevated temperatures and pesticide exposure, and to elevated temperature and drought exposure following prior pesticide exposure, contrary to our hypothesis. Even low sublethal imidacloprid concentrations reduced recruitment, despite not always affecting all fecundity measures or causing mortality. Population models showed that even small individual-level negative effects strongly reduced population growth. The 3-month field experiment overall reduced biodiversity for springtails, whereas mite diversity declined the first 6 weeks, and then recover to week 12 for most imidacloprid treatments, except the highest exposure. Watering in addition to natural precipitation had no effect neither springtail nor mites' diversity.

Our findings show that field-realistic pesticide concentrations combined with simultaneous or subsequent warming (and drought) can reduce springtail populations, and that population and species adaptation shapes the climate change dependent toxicity response, of importance for future pesticide risk assessment.

Disclaimer/Disclosure:

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2.08.T-04 Effects of Chronic Ionizing Radiation on *Arabidopsis thaliana* Populations: Evidence for Selection and Microevolution

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Radiation disasters such as Chernobyl (1986) and Fukushima (2011) cause long-term environmental contamination, raising concerns for the radioprotection of biota. Despite growing attention to ecosystem conservation, radioprotection remains largely anthropocentric. Impact assessments have mainly evaluated ionising radiation (IR) effects in a narrow set of reference species and focused on evident endpoints (e.g. mortality, morbidity, fecundity), although recent molecular studies show that doses deemed safe in natural environments can already trigger stress responses at the molecular level. We previously proposed that maintaining DNA repair and protection mechanisms in a constantly active state is energetically costly. Accordingly, transcriptome profiles of chronically irradiated plants commonly show reprogramming of oxidative stress and epigenetic pathways, but limited modulation of DNA repair genes. This suggests that (1) protective mechanisms trade off against other metabolic processes, ultimately affecting growth, fitness and, over time, population structure and evolution; (2) under sustained reactive oxygen species (ROS) production (intrinsic and IR-induced), organisms may prioritise antioxidant and epigenetic defences over high DNA-repair activity; and (3) over generations, such pressures can drive evolutionary changes that enhance adaptive responses. Here, consistent with this view, genome resequencing of populations of *Arabidopsis thaliana*, a radioresistant species, chronically exposed to IR in the Chernobyl Exclusion Zone, revealed increased nucleotide diversity, signs of a selective sweep and higher fixation rates of exonic single-nucleotide polymorphisms (SNPs). These polymorphisms accumulated in genes involved in DNA repair, cell cycle, oxidative stress and Ca²⁺/ROS signalling, and epigenetic regulation. While such microevolutionary changes may improve genome stability and stress-signalling networks under chronic IR exposure, they appear to come at the cost of seed performance and plant growth.

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2.08.T-05 Assessing Transgenerational Behavioral Responses to EE2 in *Kryptolebias marmoratus*: Implications for Neurotoxicity and Multistress Adaptation

Noémie Guirandy, Clémence Théron, Jérôme Lambert, Jean Baptiste Robert and Frédéric Silvestre, University of Namur, Belgium

Environmental stressors such as endocrine-disrupting chemicals and rising temperatures can interact to shape physiological and behavioural phenotypes across generations. Non-genetic mechanisms are increasingly recognised as potential drivers of rapid adaptation, especially when stressors persist across multiple generations. To study these processes, we used the self-fertilising mangrove killifish *Kryptolebias marmoratus*, a relevant model for studying inherited environmental effects with minimal genetic variability.

This study aimed to determine whether transgenerational exposure to 17 α -ethinylestradiol (EE2) induces behavioural effects in unexposed F2 individuals and whether ancestral EE2 exposure influences responses to a secondary thermal stressor. We examined delayed behavioural effects, potential involvement of neuronal regulatory pathways, and whether adaptive mechanisms established under EE2 exposure modulate behavioural responses when temperature is added.

Behaviour and body length were assessed at two stages in F2 generation (24 and 50 days post-hatching). The Vibrational Startle Response, in which a mechanical tap mimics sudden predation, was used to evaluate locomotor activity, startle sensitivity and resilience to fluctuating environmental cues. At 24 days, four F2 conditions were tested: control, EE2 (4 ng/L), elevated temperature (30 °C), and combined EE2+temperature. At 50 days, only control and EE2 groups were analysed. Targeted DNA methylation and mRNA expression analyses are underway to investigate regulatory mechanisms.

At 24 days, ancestral EE2 exposure did not alter behaviour, whereas temperature induced clear behavioural changes, indicating a thermal rather than EE2 effect at this stage. Body length was also affected, with distinct responses in EE2 and EE2+temperature groups, supporting both a transgenerational effect and altered responses under multi-stress conditions. At 50 days, length differences were no longer observed, but preliminary behavioural results revealed differences between EE2 and controls, suggesting delayed transgenerational effects. Ongoing molecular analyses will clarify whether epigenetic regulation contributes to these long-term outcomes.

Overall, this work provides new insight into how early exposure to an endocrine disruptor induce behavioural phenotypes across generations and influence responses to additional stressors, improving our understanding of rapid adaptive mechanisms.

2.08.P Integrating Evolution and Mechanisms to Advance Ecotoxicology Under Global Change

2.08.P-Tu082 Community-Wide Micro-Evolutionary Adaptation in Zooplankton and its Ecological Consequences

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Freshwater ecosystems are facing numerous stressors arising from climate change and anthropogenic influences. One potential mechanism through which organisms can cope with (anthropogenic) stressors is micro-evolutionary adaptation – rapid evolution occurring on a short, ecologically relevant timescale. Such rapid evolutionary change can feed back to influence ecological processes, giving rise to eco-evolutionary dynamics: the reciprocal interactions between ecology and evolution. While most previous research in the field of eco-evolutionary dynamics has focused on single species, we adopt a community-level perspective.

In order to investigate whether Cladocera (water fleas) are able to rapidly adapt to salt stress and whether this is species-specific, we performed an evolutionary selection experiment in outdoor mesocosms and a follow-up laboratory common garden survival experiment. Three different species (*Daphnia pulex*, *D. galeata* and *Simocephalus vetulus*) were either exposed to a gradually increasing salt concentration (up to 3.2 g NaCl/L) or a control treatment in fresh water in the mesocosms, either in a single-species or a community context. At the end of the experiment, laboratory clonal lines were started by selecting individuals from each of the mesocosms. With those salt-exposed or salt-naïve clonal lines, we performed a common garden experiment in which the clonal lines were exposed to a daily increasing salt concentration over a period up to 6 days – until dying. Each day, survival was determined by counting the number of individuals still alive. Results showed that for each of the three species, salt-exposed clonal lines survived longer (i.e. to higher salt concentrations) than salt-naïve clonal lines, indicating micro-evolutionary adaptation. Besides, species-specific differences in salt tolerance and the extent of adaptation were shown. A follow-up mesocosm experiment aims to investigate the effect of micro-evolutionary adaptation to salt stress on community composition and ecosystem functioning. This is of great importance to understand and predict the long-term effects of human-induced stressors on ecosystems in a rapidly changing climate.

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2.08.P-Tu083 Multiple stressor exposure in Arctic zooplankton during the polar night: Applications for ecological risk assessment

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The Arctic Ocean is experiencing accelerated rates of warming and acidification, up to four times the global average, compounded by pollutant releases from expanding oil exploration activities. These anthropogenic stressors can elicit maladaptive physiological responses, elevating mortality risks for Arctic species, particularly during the vulnerable overwintering period in the Polar Night. In this presentation, we present empirical data on the individual and interactive effects of multiple stressors, including ocean warming (+4 to +5°C), acidification (ΔpH -0.1 to -1.5 units), and contaminants such as polycyclic aromatic hydrocarbons (PAHs; 100–200 nM) and microplastics, on key Arctic zooplankton species during overwintering. We will show preliminary data on critical role of exposure timing in modulating stressor interactions and reveal potential tipping points. We elucidate underlying physiological mechanisms (e.g., metabolic disruption and oxidative stress) and genetic responses (e.g., altered gene expression in stress-response pathways) driving these effects. To advance environmental toxicology and chemistry in polar ecosystems, we propose a conceptual framework for integrating Polar Night dynamics into future multi-stressor studies, with applications for refining ecological risk assessments of Arctic biodiversity under ongoing climate and pollution pressures.

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2.08.P-Tu084 Integrating Biomarker and Mechanistic Evidence to Assess Antibiotic Toxicity under Global Change Scenarios

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Aquatic ecosystems are increasingly exposed to multiple stressors, including rising temperatures, pH fluctuations, and antibiotic contamination [e.g., sulfamethoxazole (SMX) and trimethoprim (TRIM)]. These factors interact in complex ways, altering antibiotic toxicity and disrupting key biological processes in aquatic organisms. However, their combined effects remain poorly understood, particularly under climate change conditions. This study investigated the chronic effects of environmentally relevant concentrations of SMX (150 µg/L), TRIM (30 µg/L), and their mixture (MIX: 150 µg SMX/L + 30 µg TRIM/L) on *Danio rerio* juveniles under different abiotic scenarios: temperature (26, 28, and 32 °C), pH (6.5, 7.5, and 9.0), and a combined climate-change scenario (28 °C + pH 9.0). A multi-biomarker approach, encompassing oxidative stress, lipid peroxidation, cholinergic activity, energy metabolism, and DNA damage, was used to assess toxicity and *D. rerio* health impacts. SMX showed marginal temperature-dependent toxicity but was strongly influenced by pH, causing severe effects under acidic conditions and moderate/major alterations at neutral and alkaline pH. TRIM displayed low overall toxicity, decreasing under warming but increasing with pH, leading to oxidative stress, lipid peroxidation, and neurotoxicity. MIX was generally more harmful than individual antibiotics under standard conditions, with moderate toxicity under warming and pH-dependent responses. Acidic and alkaline pH induced oxidative stress and lipid peroxidation, while neutral pH caused pronounced DNA damage. Under the combined climate-change scenario, SMX and TRIM toxicity intensified, causing severe disruptions such as lipid peroxidation and DNA damage that compromised *D. rerio* health, whereas MIX toxicity was slightly lower, suggesting compensatory or adaptive interactions during co-exposure. Overall, temperature and pH significantly modulated antibiotic toxicity by altering chemical bioavailability and physiological responses. Combined stress conditions further intensified adverse effects, emphasizing the interactive influence of chemical pollution and climate-driven stressors. These findings highlight the importance of considering multiple, co-occurring environmental stressors in ecotoxicological research and advocate for integrative approaches that better reflect realistic environmental conditions, supporting accurate ecological risk assessments and effective protection of aquatic ecosystems.

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2.08.P-Tu085 Unraveling coral sensitivity to desalination antiscalants and elevated salinity: A physiological and biochemical assessment

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Desalination is a critical source of freshwater in the Red Sea region, which hosts the highest density of desalination plants globally, filtering approximately 3.4 million cubic meters of seawater per day. Seawater reverse osmosis (SWRO) is widely regarded as the most sustainable and efficient desalination technology. SWRO filters seawater through semi-permeable membranes to produce freshwater while generating a hypersaline concentrate; brine. To prevent scaling on these membranes, antiscalants typically composed of polymers and polyphosphates are added. Brine and antiscalants are often discharged untreated, yet their behaviour in seawater remains poorly understood, raising concerns about their persistence, toxicity and impact on benthic organisms. This study aims to evaluate the isolated and combined effects of a phosphonate-based antiscalant (DTPMP-Diethylenetriaminepenta(methylenephosphonic acid)) and elevated salinity on the physiology and gene expression of the Red Sea coral *Acropora arabiensis*. Coral fragments were exposed to five concentrations of DTPMP, five salinity levels or both stressors simultaneously in a 7-day controlled ex-situ experiment. Coral photophysiology was investigated through measurements of the maximum quantum yield of

Photosystem II (Fv/Fm) to assess photosynthetic efficiency, calculation of the Normalised Difference Vegetation Index (NDVI) from coral reflectance, and rapid light curves to estimate the maximum electron transport rate (ETR_{max}) as a proxy for photosynthetic capacity. Water quality parameters (nutrients, ammonium and total alkalinity) were monitored throughout the study, and coral tissues were collected at the end of the experiment for transcriptome-wide gene expression. Preliminary results showed that DTPMP alone had no significant effect on Fv/Fm, NDVI, or ETR_{max}. Elevated salinity caused a significant decline in Fv/Fm and NDVI, while no interactive effect between DTPMP and salinity was observed for any of the three parameters. Transcriptomic analyses are ongoing and will elucidate whether cellular stress pathways are activated prior to changes detectable through photophysiology. This work provides the first mechanistic assessment of coral responses to phosphonate antiscalants under realistic hypersaline scenarios and contributes to environmental risk evaluation of desalination activity in coral-dominated ecosystems.

2.08.P-Tu086 Animal Organ Size Database: A Resource for Toxicology Research

Félix Leiva, Luke Ockhuijsen, Jasmijn Polinder, Louise Schreyers, Jie Xiong and Jan A.J. Hendriks, Radboud University, Netherlands

After substances are released into the environment, they become available to organisms. The metabolism of these substances in animals, particularly vertebrates, is studied *in silico* using Physiologically Based Kinetic (PBK) models. These models simulate absorption, distribution, metabolism, and excretion through organ compartments. Currently, PBK models mainly focus on adult humans, rats, and a limited number of other species, and they require species- and substance-specific parameters that depend on how substances are distributed across different organs. A key parameter in this context is size, as many physiological functions, such as organ perfusion, may inevitably be influenced by organ size. However, comprehensive risk assessment requires broader coverage across taxa, species, and organs, which poses challenges for developing generalized PBK models. To address this gap, we developed a systematic map and compiled an extensive database from literature of paired organ and body size measurements. This database comprises over 10,000 records from 366 species spanning eight animal classes. It provides size estimates for 53 organ types organised into ten physiological systems, with most data derived from the digestive, circulatory, and excretory systems. In addition, extensive metadata are included to contextualise the original studies. This database enables (i) exploration of interspecific variation patterns and derivation of interspecific regressions linking organ metrics to body size, ecological traits, and habitat; ii) examination of intraspecific scaling relationships, incorporating variables such as sex to assess whether males and females differ. In vertebrates, sexual dimorphism can influence these scaling relationships, while shared evolutionary history (phylogeny) introduces non-independence among species that must be accounted for in comparative analyses; and iii) integration with other traits, such as organ-specific perfusion and metabolic rates. Finally, due to its flexible metadata structure, the database can be expanded to include the effects of chemical exposure on organ size itself. Altogether, this comprehensive and reproducible resource provides a robust foundation for improving the parameterisation and cross-species applicability of simulation models based on physiological and kinetic principles, thereby enhancing understanding of how substances accumulate in organisms and the underlying mechanisms, particularly those related to organ size.

2.09 Integrative and Mechanistic Approaches to Wildlife Ecotoxicology: Cross-Species Variability and One Health perspective

2.09.T-01 Sublethal Anticoagulant Rodenticide Exposure Drives Systemic Stress Signatures in the Eurasian Eagle-Owl (*Bubo bubo*): Insights from blood RNA-Seq

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Anticoagulant rodenticides (ARs) inhibit VKORC1 and disrupt the vitamin K cycle, but sublethal exposure in predators can also provoke oxidative, metabolic and immune disturbances. Raptors are especially vulnerable due to their trophic position and repeated ingestion of contaminated prey. The Eurasian eagle-owl is a key sentinel species, yet the systemic molecular effects of environmentally relevant AR exposure remain poorly characterised. Here, we used whole-blood RNA-seq combined with EchoGO, a cross-species enrichment

framework, to resolve transcriptional pathways affected by AR exposure in wild nestlings.

Blood samples from chicks with high and low VKORC1 expression and detectable AR residues were analysed using DESeq2 (FDR < 0.05). Goseq was used for bias-aware enrichment, and EchoGO integrated Goseq with g:Profiler across eight avian reference species, generating strict and exploratory consensus terms. Semantic reduction (RRVGO) summarised pathway-level responses, and a mechanistic AR-toxicity panel (predefined from the literature) was used to test expected physiological axes, including coagulation, vascular integrity, xenobiotic metabolism, unfolded-protein response, immune activation and energy balance.

EchoGO revealed a multi-systemic stress architecture consistent with sublethal AR exposure. Key high-level terms included blood circulation, defence response to virus, antigen presentation via MHC I, positive regulation of type II interferon response, macromolecule biosynthesis, pyruvate metabolism and intracellular potassium homeostasis. These clusters collectively indicate vascular remodelling under VKORC1 blockade, ER-stress and proteostasis responses, metabolic and redox reprogramming, and strong innate immune activation dominated by interferon-stimulated genes (e.g., RSAD2, OAS2, STAT1, IRF3, STING).

The a priori mechanistic panel was fully validated: all predicted parent categories were significantly represented in EchoGO consensus outputs. Cross-species evidence (especially from *Aquila chrysaetos*, *Gallus gallus* and *Serinus canaria*) highlighted conserved immune-metabolic signatures across birds.

Overall, blood transcriptomics provided a non-lethal window into systemic AR impacts in a wild raptor. These results support the use of OMICs-based, cross-species frameworks for biomonitoring and for improving mechanistic understanding of chemical stress in wildlife under a One Health context.

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2.09.T-02 Mechanistic Effect Models in Wildlife Risk Assessment: Insights from 15 years of Application *Oliver Jakob¹, Torben Wittwer¹, Lara Ibrahim¹, Martin Vallon¹, Finn Bastiansen¹, Felix von Blanckenhagen² and Jan-Dieter Ludwigs², (1)RIFCON GmbH, Germany, (2)Rifcon GmbH, Germany*

Mechanistic effect models (MEMs), including population models, are increasingly recognized as higher-tier tools in environmental risk assessment for wildlife because they link individual-level effects to population-level outcomes under realistic scenarios. Despite being mentioned in relevant guidelines for nearly two decades, their practical implementation has faced significant hurdles. Nevertheless, MEMs provide ecologically relevant insights beyond standard laboratory tests and support regulatory decision-making by providing weight-of-evidence data, guiding field study design, and evaluating mitigation measures. Overall, they are and will remain a valuable tool for reliable assessments in the approval process.

This presentation summarizes nearly 15 years of experience applying population models to wildlife in agricultural landscapes. We highlight three key applications using eVole, an individual-based, spatially explicit simulation model for common voles (*Microtus arvalis*): (i) informing risk assessments through simulations of long-term population responses under conservative environmental and exposure scenarios, (ii) supporting field studies design and interpretation by comparing habitat-specific population dynamics and (iii) evaluating mitigation strategies such as precision pesticide applications. We also share insights from stakeholder discussions and regulatory evaluations, concluding with key advantages and remaining challenges.

Our review shows that population modelling can (i) identify population-level risks posed by pesticides and quantify margins of safety, (ii) assess habitat-specific population dynamics to support conservative field study designs, and (iii) explore precision application patterns to better understand how exposure fragmentation interacts with average activity ranges of farmland species. These findings highlight the potential of MEM applications to complement empirical approaches and enhance ecological realism in pesticide risk assessments. Beyond current advances, future adoption in regulatory frameworks will benefit from standardized processes, transparent documentation, and active stakeholder engagement. MEMs offer cost-effective, powerful tools for advancing pesticide risk assessment and management by integrating ecological complexity and supporting informed decisions. With regulatory willingness, modelling – long referenced in wildlife guidelines and validated through practice – can readily evolve into a standard procedure.

2.09.T-03 Multi-Level Impacts of Fungicide Exposure on Red-Legged Partridge

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Triazoles are a group of fungicides applied as seed coating treatments which pose a potential threat to granivorous birds. Experimental studies have shown that triazoles may impair avian reproduction by reducing fertilization rate and brood size even if exposure occurs months earlier, but the mechanism behind these effects remains unknown. Triazoles inhibit lanosterol-14 α -demethylase, a key enzyme in fungal cell wall synthesis which also plays an essential role in endogenous cholesterol synthesis in animals. Triazole exposure have been shown to reduce cholesterol levels and alter gene expression linked to its synthesis. Disturbances at this step can affect the biosynthesis and regulation of steroid hormones, derived from cholesterol, potentially causing structural and functional alterations in endocrine organs and compromising reproductive success. This study evaluates the reproductive effects of Raxil Plus on red-legged partridges and explores how triazoles may generate long-term reproductive impacts by through gonadal and molecular alterations. Partridges were fed with wheat seeds treated at 0%, 20% or 100% of the labelled application rate (n= 38 per treatment) for 21 days in late autumn, and reproductive parameters were monitored in spring. In addition, 6 partridges (3 males and 3 females) from each treatment were euthanized after exposure (December), and at the beginning (March), during (June) and the end (July) of the egg laying period to assess gonadal size, histopathology and expression of 13 steroidogenesis-related genes. Significant reductions in fertilization, hatching, and chick survival rates were observed in couples exposed more than 4 months before. In June, right testis length decreased in treated males, while females consistently showed larger gonads in the high-dose group across all samplings. Histological analysis showed increased atretic follicles in July, during the end of reproductive period, but highest-dose females had a significantly higher proportion of viable follicles in that sampling. In exposed males, reduced gonadal expression of PGR, ESR1, CYP19A1 and ABCA1 was detected in March. PGR showed the same pattern in females. In June, ABCA1 was overexpressed in high-dose females. Results indicate that Raxil Plus exposure induces long-term reproductive impairment through multiple physiological mechanisms, including gonadal size changes, altered follicular dynamic and molecular disruptions in steroidogenesis.

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2.09.T-04 Predicting Acetaminophen Toxicity in Protected Bird Species Based on UDP-Glucuronosyltransferase Docking Site Analysis

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Previous work has demonstrated that snake species are extremely sensitive to acetaminophen, the active compound in paracetamol. The underlying mechanism for this toxicity was determined to be the lack of phenol-type UDP-glucuronosyltransferase, and N-aminotransferase. To investigate if bird species could also be sensitive to acetaminophen, which is used to manage the invasive brown treesnake on the island of Guam, a dosing experiment was conducted with chickens. PCR analysis showed that chickens do have 4 isoforms of the glucuronosyltransferase (UGT) enzymes that were upregulated in response to the acetaminophen exposure. To explore if other bird species have UGT isoforms that can metabolize acetaminophen, liver samples were collected from a wide variety of coastal Carolinian bird species, including crow, wading birds, pelican, vulture, hawks and owls. Liver samples of 15 different species were tested for phenol-type UGT enzymatic activity and demonstrated that some birds have very good activity towards acetaminophen-like compounds, while others had no significant activity. Surprisingly, there did not seem to be a connection between phylogenetic relationship, or food preference, and the enzyme activity, which complicates extrapolation to protected species, like native Pacific Island bird species.

PCR primers for the four UGT isoforms that were upregulated in chicken after acetaminophen exposure were tested in PCR and qPCR reactions on liver samples from all other collected bird species. While PCR analysis initially suggested that some of these primers would work in all 15 species, quantitative PCR showed only results for chicken and one of the owl species. Further work was pursued to evaluate UGT isoforms in published genomes for similarity between bird and mammalian isoforms that are active towards acetaminophen. Using 3D protein folding and AI-driven docking and homology models, conserved regions in the protein sequences were identified that are essential for substrate binding. Based on these sequences universal PCR primers for UGT isoforms that are predicted to have enzymatic activity towards acetaminophen were designed and tested for use

in a larger variety of bird species, thus providing a predictive tool for acetaminophen metabolism and toxicity in all birds.

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2.09.T-05 Age-dependent variation in trophic ecology and mercury concentration in Whiskered tern (*Chlidonias hybrida*) chicks.

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Mercury (Hg) occurs naturally in the environment; however, anthropogenic activities have increased its turnover, making it more available to living organisms. In aquatic ecosystems, one of its forms, inorganic mercury (iHg), is converted to its organic form methylmercury (MeHg), which can be easily bioaccumulated in organisms and biomagnified along the food chain. In the early life of nestlings, maternal transfer is responsible for Hg exposure, whereas dietary uptake dominates as a main source of Hg in later development.

In that context, the study aimed to determine whether total Hg concentrations in blood were related to chick age. We also investigate whether foraging ecology, including trophic position (proxied by $\delta^{15}\text{N}$) and feeding habitat (proxied by $\delta^{13}\text{C}$), differs according to their age. The study was conducted in the northern part of the Upper Vistula Valley (southern Poland, Central Europe) on 224 Whiskered tern (*Chlidonias hybrida*) chicks (117 and 107 individuals in 2024 and 2025, respectively). Blood samples were collected from the same individuals every few days, from hatch until fledge or death. Total Hg concentration (Hg, hereafter) was measured in 625 samples using a cold vapour atomic absorption spectrometer (CV-AAS) with no prior mineralisation. The relative abundance of stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) was measured in 174 samples using isotope ratio mass spectrometry (IR-MS). All procedures and data representation were carried out in R 3.5.2 (R Core Team, 2018).

We found that Hg was present at quantifiable concentrations in all specimens (ranging from 0.02 to 0.71 $\mu\text{g}\cdot\text{g}^{-1}$, wet weight). After hatching, Hg concentration decreases, which suggests the impact of body dilution and deposition into growing feathers. In the middle of the growth, Hg started to increase, suggesting the influence of dietary uptake. According to trophic ecology, the $\delta^{13}\text{C}$ values (ranging from -30.60‰ to -22.42‰) remain constant during the nestling period, in contrast to $\delta^{15}\text{N}$ values (ranging from 8.03‰ to 14.15‰), where a non-linear pattern was observed. Our results suggest that despite the similar foraging habitat, a dietary shift of prey is observed during the chick growth period. Overall, the study highlights the importance of biomonitoring Hg concentrations in adult birds, especially females, as a potential main source of mercury in hatchlings. Moreover, it is worth emphasising to consider age in the dietary interpretation of isotope signatures in wild nestlings.

2.09.P Integrative and Mechanistic Approaches to Wildlife Ecotoxicology: Cross-Species Variability and One Health perspective

2.09.P-Tu087 Dried Blood Spots as a Tool for Wildlife Ecotoxicology: Insights from Bat Omics

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Understanding the impact of environmental contaminants on wildlife health requires analytical approaches that are both sensitive and logistically feasible in field settings. In this study, we explore the potential of using dried blood spot (DBS) sampling as a minimally invasive, cost-effective, and scalable tool for wildlife ecotoxicology, with a focus on bats as bioindicators of environmental pollution. DBS sampling simplifies field collection, storage, and international transport of biological material, overcoming many of the challenges associated with conventional blood sampling in wildlife research. Using DBS from multiple bat species, we demonstrate how this approach can support exposomics analyses to profile chemical exposures and metabolomics analyses to assess physiological and biochemical effects of contaminant burdens. Our findings highlight the robustness of

DBS for both targeted and untargeted analyses, offering new opportunities for large-scale, integrative monitoring of wildlife health in the context of global environmental change.

2.09.P-Tu088 Multiple Stressors in Killer Whales: Using the COVID-19 Travel Restrictions to Explore the Effect of Whale Watching, Contaminants and Nutritional Status

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Marine mammals are continuously exposed to multiple stressors, however, risk assessments often address only single stressors and underestimate combined effects. Killer whales (*Orcinus orca*) from Norway have high concentrations of contaminants. In addition, they face frequent boat traffic, as winter whale watching has increased dramatically since 2012. Contaminants and boat traffic, as well as fluctuations in prey availability, can cause physiological and nutritional stress, possibly affecting health. This study assessed the effect of multiple stressors on Norwegian killer whales by steroid hormone analysis and transcriptomics. Blubber and skin biopsy samples were obtained from 88 photo-identified individuals of varying age and sex. Samples were taken in both seasons of low boat traffic (spring/summer months and winter 2020, with reduced boat traffic due to COVID-19 travel restrictions) and high boat traffic (winters 2019, 2021 and 2022). Five of the 88 individuals were sampled in both low and high boat traffic seasons. Cortisol, cortisone, progesterone, testosterone and androstenedione were quantified by liquid chromatography-tandem mass spectrometry, and 28 health-related mRNA transcripts were additionally evaluated. Legacy and emerging contaminants, fatty acids, stable isotopes, and mercury were also analysed from the same biopsy samples to provide information on contaminants, nutritional status, and diet. We found no effect of boat traffic on cortisol levels across all whales, however, all re-sampled whales had higher cortisol levels in the high boat season. This highlights that population-level averages may hide individual responses. We found a positive correlation between pollutants and cortisol, and a subtle but significant synergistic effect between contaminants and boat traffic. Furthermore, genes typically expressed in response to stress and the biotransformation of contaminants were more strongly associated with whales that had been sampled in high boat traffic seasons. Female killer whales appeared to have a stronger response to contaminants than males, and a higher rate of lipid catabolism in winter seasons, possibly due to reproduction. This is the first investigation of the interaction between contaminants, nutritional stress, and boat traffic, and can aid the establishment of protective conservation and management strategies.

Disclaimer/Disclosure:

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2.09.P-Tu089 First report of anticoagulant rodenticides and their impact in birds of prey from Northern Patagonia, Argentina

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Recent studies have shown that the second-generation AR (SGARs) can induce coagulopathy in terrestrial raptors at very low concentrations. The Andean region of northern Patagonia (Argentina) represents a critical scenario for this threat, as it is the epicentre of a deadly hantavirus transmitted by wild rodents, which in turn undergo cyclic population outbreaks due to masting of native bamboo. This has triggered the massive application of ARs in peridomestic and institutional settings. High mortality in American barn owls (*Tyto furcata*) has been associated with some of these episodic rodent outbreaks, but an association with ARs has not yet been tested. In the present study, birds of prey (n=44) from a total of 12 species were collected dead or

moribund in northern Patagonia between 2012 and 2025. The details of the collection site, the clinical signs, the necropsy findings, including the presence of haemorrhages and non-clotted blood, the body condition and the suspected cause of death were recorded. Liver samples were collected analysed using liquid chromatography combined with tandem mass spectrometry. Anticoagulant rodenticides were detected in the liver of avian scavengers and in diurnal and nocturnal birds of prey. In total, 6 out of the 12 studied species showed AR residues in liver, and this included individuals of 4 families of birds with an overall prevalence of 18.1%. The prevalence of SGARs was 33.3% in the avian scavengers, 18.2% in the diurnal raptors and 16.7% in the nocturnal raptors. The most frequently detected SGAR was brodifacoum (17.8%), followed by bromadiolone (8.9%) and difenacoum, flocoumafen and difethialone (2.2%). The prevalence of SGARs did not differ between urban, rural and natural areas nor between the three groups of birds (scavengers, diurnal and nocturnal raptors). The highest prevalence was observed in western Patagonia (but not significantly). The two individuals with the highest concentration of SGARs in liver were a Magellanic horned owl (*Bubo virginianus magellanicus*) with 1119.2 ng/g and a rufous-legged owl (*Strix rufipes*) with 267.3 ng/g. In both cases, field and necropsy findings and the reduced vitamin K1 levels indicated that these birds may have died by SGAR poisoning. This is the first report of the presence of SGARs in birds of prey from Argentina, which confirms the suspects of the potential impact that these biocides may have on the conservation of South American wildlife.

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2.09.P-Tu090 New Approach Methods (NAMs) offer opportunities for hazard assessment in species at risk

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Species at risk (SAR) may be disproportionately affected by environmental contaminants, but demonstrating this impact can be difficult. One key challenge is that chemical hazard data are rarely available for ecologically vulnerable species. International efforts to develop New Approach Methods (NAMs) for toxicity testing - which aim to replace, refine, or reduce animal use - may offer solutions. NAMs are typically high-content and animal-free, making them well-suited for application to SAR. In this talk, I will review our recent experiences developing NAMs for fishes and birds, emphasizing our work on vulnerable species. In one study, we generated transcriptomic points of departure (tPODs) for the tire-wear transformation product, 6PPD-Quinone, in six different fishes including two listed species of the genus *Moxostoma*. These data helped us to identify outlier species that have poor conservation status and the potential for heightened sensitivity to this chemical. In another study, we used in vitro and in silico approaches to identify avian species that may exhibit heightened sensitivity to dioxin-like compounds. Our investigations focus on two avian orders: Psittaciformes (parrots) and Procellariiformes (petrels and albatrosses). Collectively, our experiences suggest that NAMs can facilitate hazard assessment for species at risk. However, selecting an ecologically vulnerable species over a standard laboratory model requires careful consideration of the trade-offs involved.

2.09.P-Tu091 Transcriptome analysis in free-ranging wood mice under a chronic exposure to a mixture of trace metal

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Anthropization is known to negatively impact the environment, notably through the release of harmful contaminants such as trace metals (TMs). The impacts of some TMs such as Cd and Pb on the immune system and oxidative stress response are well known in laboratory based models. These models exhibit significant differences with wild animals as they do not face similar challenges and consequently poorly represent wild animals physiological response to TMs exposure. To address this gap, we investigated the effects of a chronic exposure to TMs on free ranging wood mice (*Apodemus sylvaticus*) on the immune system at the gene expression level. Wood mice were captured following a well characterized pollution gradient in the vicinity of a former smelter including three levels of contamination: contaminated, intermediate and control. The liver and kidneys were used to measure the levels of TMs in the individuals (ICP-MS and ICP-AES). Blood samples were used to evaluate the antioxidant response biomarkers using Randox® automated assays. RNA extracted from the

spleen was sequenced with Novogene in order to assess the status of the immune system across groups. Both Pb and Cd concentrations were significantly higher in the contaminated group than the control group. Glutathione peroxidase (GPx) concentration was also significantly increased in the contaminated individuals, indicating a rise in oxidative stress response consistent with previous studies on wild mammals. Differentially expressed genes we found to belong to five different categories including: B-cell mediated immunity, haematopoiesis, immunity and oxidative stress and Ca²⁺ dependent. Overall, most genes were found to be suppressed in the individuals from the contaminated area and activated in the control area, which could suggest the potential suppression of key immune mechanisms. Several Ca²⁺ genes were also found to be suppressed in the contaminated group which is consistent with known interference of Pb in calcium dependant functions. Our study shows that chronic exposure to a mixture of Cd and Pb increases the oxidative stress response and impacts the immune system in free-ranging wood mice. These results highlight the need for the development of techniques applicable to wild species for accurate protein quantification (e.g cytokines) to complement transcriptomic data thereby improving our understanding of how environmental contaminants can modify the physiology and affect the health of wild mammals.

2.09.P-Tu092 Linking Mercury and Reproductive Performance in Grey Seals (*Halichoerus grypus*)

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Mercury is a heavy metal and global contaminant that previous studies have linked to deleterious effects on wildlife health, survival, and reproduction. Significant knowledge gaps remain in the research regarding how mercury toxicity in individuals may eventually compound, leading to population impacts in wildlife. It is unclear how maternal mercury load in individuals directly impacts maternal reproductive performance and offspring fitness. As a part of a larger project exploring relationships between total mercury concentrations ([THg]) in tissues and reproductive performance in female grey seals and their pups, here we present on (1) intra- and interannual variation in adult female [THg] and (2) connections between adult female [THg] and pup fitness characteristics, such as birth or weaning mass. Understanding intra- and interannual variation in adult female [THg] will aid in identifying dynamics underlying mercury transfer in marine food-webs, while links between adult female [THg] and pup fitness characteristics will help identify potential mechanisms linking mercury and population level dynamics. From 2010-2013, vibrissae samples (n = 211) from breeding adult female grey seals and their pups were collected in conjunction with a long-term monitoring project tracking individuals over time at two locations in Scotland (Isle of May and North Rona). Reproductive data were also collected including adult female and pup mass both near birth and at weaning. Whiskers were sequentially sectioned from the root to the tip and analyzed for [THg] (ppm). As whiskers represent approximately one year of growth, intra-annual patterns in [THg] were examined to characterize individual seasonal patterns in mercury and health risk. Interannual [THg] were found by utilizing whiskers repeatedly collected from the same individual across multiple breeding seasons. Preliminary data revealed considerable intra- and interannual variation in [THg] among individual adult females, with some individuals reaching levels of moderate risk. Future results will continue to shed light on how maternal mercury load may directly impact pup fitness characteristics or maternal reproductive efficiency, and how these impacts may contribute to population level effects in a wild system. These data become more meaningful as a rapidly changing climate impacts marine ecosystems, the species that occupy them, and the humans that rely on them for subsistence or traditional resources.

Disclaimer/Disclosure:

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2.09.P-Tu093 Anticoagulant Rodenticides in Birds of Prey from Switzerland - A combined approach of chemical analysis and pathological examinations

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Anticoagulant rodenticides (ARs) enter the environment due to their widespread use for rodent control. These substances, particularly second-generation ARs (SGARs), possess persistent, bioaccumulative, and toxic (PBT) properties that can result in the secondary poisoning of non-target organisms. In this context, the exposure of birds of prey to ARs is well-documented internationally. Recently a total SGAR liver toxicity threshold of 10 ng/g has been proposed.

The current study aimed to expand the monitoring of AR exposure in Switzerland and combined it with postmortem examinations of birds of prey. A total of 21 common kestrels (*Falco tinnunculus*) and 82 common buzzards (*Buteo buteo*) were sampled from April 2024 to February 2025. Fresh carcasses were submitted from wildlife rehabilitation centers after birds died during rehabilitation or needed to be euthanized. The carcasses were examined macroscopically to determine the cause of death and categorized as follows: "common trauma", "AR toxicosis", or "other". A liver sample and, if possible, a blood sample were analyzed for residues of seven SGARs.

Only 4% of the birds were diagnosed with suspicion of "AR toxicosis", while 30% were categorized as "common trauma". The majority of the examined birds (66%) were categorized as "other". Histopathology was performed on ten birds with different death causes but no severe internal bleeding, as would be expected from AR toxicosis, was found. 84% of all examined liver samples showed SGAR residues above the sample-specific LOQ. Total residue concentrations ranged from 0.1 to 582 ng/g. 46% of the samples had concentrations above the proposed threshold of 10 ng/g. 15% of the concentrations exceeded 100 ng/g. 69% of the bird livers examined in this study contained two or more ARs, and nearly 10% contained up to five different ARs.

This study found no link between the diagnosis "AR toxicosis" based on postmortem examinations and AR-concentrations measured by chemical analysis. However, the study confirmed widespread and, in some cases, high background contamination of ARs in Swiss birds of prey. Since 86% of the animals studied were affected, the influence of sublethal effects should be considered. These can include reduced general fitness and impaired breeding success, both of which may negatively impact overall survival. Since ARs are PBT substances that are regularly found in wildlife, the overall focus should be on replacing them with less problematic substances.

2.09.P-Tu094 Mapping Anticoagulant Rodenticide Binding Across Chordata VKORC1 Via Molecular Docking: Revealing Taxonomic Patterns And Outliers

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Anticoagulant rodenticides (AR) control rodents through VKORC1 inhibition, but may harm non-target species. Regulatory in vivo tests use limited representative species, overlooking species sensitivities. Computational docking assesses AR-VKORC1 binding affinity (BA) and ligand position across species, offering mechanistic insight and reducing animal use. We hypothesized that docking with high taxonomic resolution could provide detailed in silico risk assessments by evaluating susceptibility across species and identifying outlier species. First, we searched for protein sequences homologous to brown rat VKORC1 (SeqAPASS v9) and found 2166 initial results. Many sequences were predicted, partial or inconsistently annotated, often corresponding to VKORC1-like1, and after an essential curation we kept 760 complete VKORC1 sequences. Then, we selected among the Chordata all those sequences from the least represented classes and those with >80% similarity among the most represented, resulting in 101 species belonging to six classes (4 Actinoterygii, 7 Amphibia, 2 Ascidiacea, 2 Aves, 65 Mammalia and 21 Reptilia). After sequence comparisons analysis among species, we obtained 76 unique VKORC1 sequences. Then, we performed blind docking of nine AR (AutoDock Vina v1.1.2) to analyze binding positions and taxonomic patterns in AR-VKORC1 interactions. Second generation AR showed the highest BA, ranked as flocoumafen, brodifacoum, difenacoum, difethialone and bromadiolone (-12.62±0.96 to -10.52±0.53 kcal/mol), over the first generation AR diphacinone, chlorophacinone, coumatetralyl and warfarin values (-10.31±0.63 to -8.86±1.27 kcal/mol). High-BA conformations occupied the central 3D region, with a gradient of decreasing affinity outwards. Clear taxonomic patterns emerged as mammals formed a compact cluster, and amphibians overlapped with reptiles and birds, the latter separating into Galliformes and Columbiformes. Also, Actinopterygii and Ascidiacea formed distinct groups, with Ascidiacea separating into two clusters consistent with Enterogona and Phlebobranchia. Several species-ligand combinations showed outlier behavior, including *Mus caroli*, *Suricata suricatta* and *Sigmodon hispidus* (Mammalia), *Xenopus laevis* (Amphibia) and *Gallus gallus* and *Caloenas nicobarica* (Aves). Outlier species may exhibit distinct interactions with key active-site residues, suggesting AR effects could differ from related species and highlighting the need for species-specific assessment.

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2.09.P-Tu095 New Approach Methodologies to Assess Rodenticides Effects: Application of Transcriptomics in a Rat (*Rattus norvegicus*) Hepatoma Cell Line after Exposure to Difenacoum

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The brown rat (*Rattus norvegicus*) is a globally distributed species and one of the most significant urban pests, frequently associated with public health risks. Its remarkable metabolic adaptability has facilitated the development of resistance to anticoagulant rodenticides (ARs). This resistance can be provoked by mutations in the *vkorc1* gene, encoding the vitamin K epoxide reductase complex subunit 1. However, growing evidence suggests that additional molecular mechanisms, including detoxification and redox pathways, may contribute to tolerance and metabolic adaptation. Understanding these mechanisms is essential to improve toxicological assessments for cross-species extrapolation and to refine environmentally responsible pest management strategies under the One Health paradigm. In this context, the application of New Approach Methodologies (NAMs) allows generating extensive information with important savings of time, resources and experimental animals.

We hypothesized that exposure to ARs would activate metabolic and stress-related pathways beyond the classical vitamin K pathway. In order to explore these additional molecular responses, we analysed the transcriptomic profile of the rat hepatoma cell line (H4IIE) exposed to sub-lethal concentrations of the second-generation AR difenacoum. Control and treated cells were analysed by RNA sequencing and processed using an integrative online bioinformatics pipeline (RaNA-seq). Differential gene expression was assessed using DESeq2 (Wald test), identifying 252 differentially expressed genes (DEGs) between both conditions. Functional enrichment analysis of gene ontology (GO) terms and metabolic pathways indicated upregulation of genes related to drug response, transmembrane transport and ROS biosynthesis, indicating activation of detoxification and oxidative stress defence mechanisms. Conversely, mitochondrial functions such as oxidative phosphorylation and ATP synthesis were downregulated, suggesting impaired energy metabolism and mitochondrial dysfunction.

Overall, these in vitro approaches provide novel insights into the molecular response of *R. norvegicus* to AR exposure. The OMICs-based findings highlight candidate genes linked to metabolic resistance and enhance understanding interspecies variability, supporting more predictive, One Health-oriented risk assessments.

Keywords: anticoagulant rodenticides, *vkorc1*, transcriptomic, New Approach Methodologies, metabolic adaptation, One Health.

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2.09.P-Tu096 Exposure to Anticoagulant Rodenticides Along the River Gradient: A Case of Study of the American Mink in Eastern Spain.

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The American mink (*Neovison vison*) is a semi-aquatic medium-sized carnivore of Mustelidae family that became an established alien species in Europe during the last decades.

This species is an opportunistic and generalist predator which typically preys on fish and amphibian species close to rivers, lakes, or dams, but also can feed on birds or small mammals such as rodents or lagomorphs. In this study, we evaluated the exposure to anticoagulant rodenticides in 48 American mink specimens captured along the Palancia river (Castellón, Valencia Autonomous Community, eastern Spain). We further evaluated urban and agricultural relationships of ARs exposure. The main objectives of this study were to: 1) quantify the levels of exposure in American mink individuals from eastern Spain, 2) evaluate variation of AR exposure along a riverine gradient, 3) evaluate the relationship between environmental variables and anticoagulant rodenticide

exposure, 4) assess how different spatial scales may influence the factors determining ARs exposure and, 5) estimate the intensity of exposure to these compounds in continental water masses in Spain. American mink specimens came from direct captures on trapping platforms located along the Palancia river. The individuals were necropsied after death, and liver samples were kept frozen until procedure. ARs were extracted using an adapted technique of QUECHERS, and quantified using a LC-MS-qTOF equipment.

The study demonstrated that anticoagulant rodenticides were present in the aquatic environment and that some of the captured American mink individuals may have suffered effects derived from acute intoxication to SGARs. Additionally, given the ongoing control and eradication programmes targeting American mink in Spain, we propose this species as an appropriate sentinel for monitoring AR exposure in continental freshwater systems.

The American mink is an invasive species in Spain but also is the main competitor of the European mink (*Mustela lutreola*), a native species recently classified as one of the most endangered mammal species in the world. The great niche overlap between both species has contributed to the severe decline of the latter. However, our findings provide valuable insights in the conservation of the European mink, as the threats currently affecting American mink populations may be impacting the remaining European mink populations.

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2.09.P-Tu097 Biomonitoring of Heavy Metals in Non-Commercial Elasmobranchs for Human Consumption in Southeastern Spain

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Heavy metals are a threat to both marine and terrestrial ecosystems, including humans. In the case of elasmobranchs, their high trophic position and prolonged longevity make them particularly susceptible to bioaccumulation of these pollutants. In certain scenarios, the fishing exploitation of these species can pose a risk to public health. The objectives of this study were: 1) to evaluate the degree of exposure to heavy metals in non-commercial sharks caught in the Murcian coast, 2) to evaluate the possible consequences on the health of these species and 3) to estimate the potential risk to the human population if they were to be consumed. The concentrations of certain heavy metals (Hg, Cd, Pb, As and Se) were quantified in liver and muscle of individuals non-marketable of seven species shark and two species rays, caught off the coast of the Region of Murcia during the period 2010-2024. Correlations between tissue concentrations of metals were also examined, as well as the influence on exposure of variables such as gender, the taxonomic group and the area from which they come. All concentrations were expressed in wet weight (w.w.). The maximum concentrations of Hg in liver were detected in sharks (14.42 ± 16.44 mg/kg, w.w.), while in muscle they were found in rays (4 ± 7.99 mg/kg, w.w.). As for Cd, the highest concentrations in liver were found in sharks (1.25 ± 2.04 mg/kg, p.h.). In muscle samples, neither Cd nor Pb was detected. The latter metal was only residually found in one liver sample. As was found in higher concentration in shark liver (20.66 ± 17.63 mg/kg, p.h.) and in ray muscle (41.90 ± 30.87 mg/kg, p.h.). Finally, Se was found in higher concentrations in both liver (7.61 ± 7.3 mg/kg, p.h.) and muscle (1.38 ± 3.1 mg/kg, p.h.) of sharks. Significant differences in Cd and Se concentrations were observed between tissues, with higher concentrations found in liver tissue. Significant differences were also observed between groups, with higher Cd concentrations in sharks. The levels of metals in sharks did not differ according to area and sex. Regarding the risk to public health that these heavy metals may present, more than 75% of the specimens sampled showed muscle concentrations of Hg above the legal limits established for human consumption.

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2.09.P-Tu098 What's on the Menu? Deciphering Rodenticides' Trophic Transfer to the Aquatic Top Predator Eurasian otter (*Lutra lutra*)

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Anticoagulant rodenticides (ARs) such as brodifacoum, difenacoum, and bromadiolone are widely applied to control commensal rodents. Their frequent use in sewer systems and along riverbanks creates direct pathways for emissions into water bodies, thereby increasing the risk of unintended AR exposure of aquatic wildlife. Correspondingly, hepatic AR residues have been detected in fish and at even higher concentrations in upper-trophic predators, indicating not only bioaccumulation but also potential biomagnification. However, exact exposure routes of biota in aquatic ecosystems, as well as specific trophic pathways involved, remain poorly understood. This study aims to assess the exposure of Eurasian otters (*Lutra lutra*) in Germany to approved first- and second-generation ARs by identifying trophic pathways that may mediate the transfer of these contaminants within aquatic food webs. We hypothesise that specific taxonomic prey groups may promote trophic transfer of ARs, and that otters specialising on prey of higher trophic levels may exhibit elevated AR burdens due to biomagnification. To address this research question, otter carcasses (n = 121) were collected across Germany between 2007- 2025, and liver tissues and intestinal contents were sampled. AR residues were quantified in liver samples by liquid chromatography - tandem mass spectrometry, while diet composition was reconstructed through metabarcoding of gut contents combined with hard-part analysis of stomach contents. This study contributes to understanding the behaviour and exposure pathways of ARs in aquatic environments, thereby providing important information concerning the risks for wildlife, as well as guidance for future monitoring and risk mitigation efforts.

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2.09.P-Tu099 Environmental RNA metabarcoding enables community-level risk assessment based on response of benthic communities

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To achieve the biodiversity restoration, it is important to identify the impact of chemicals on ecosystems and strategically reduce their risks. The effects of chemicals on ecosystems in aquatic environments has generally been predicted using the assessment factor (AF) method, in which the toxicity data for test organisms is extrapolated to calculate the predicted no-effect concentration (PNEC). However, contaminated aquatic ecosystems are affected by both the direct toxic effects of chemicals on single organisms and indirect changes via ecological interactions; thus, the results of the hazard assessment methodologies that are based on extrapolation from toxicity tests reflecting only the direct effects of chemicals are likely associated with large uncertainties. The development of community-level assessments that consider complex ecological interactions may contribute to the accurate visualization of the effects of chemical contamination on ecosystems. Therefore, we focus on the environmental RNA (eRNA) and the environmental DNA (eDNA) released by macroorganisms in aquatic environments to directly capture the responses of biological communities.

In this study, eRNA/eDNA metabarcoding was performed in a mountain stream receiving linear alkylbenzene sulfonate (LAS) effluent from a chemical plant to assess the effects of LAS on ecosystems by comparing the biological communities at sites with different levels of LAS contamination. This approach revealed that eRNA can capture the response of benthic communities to LAS more sensitively than eDNA, and that the effects of LAS contamination on ecosystems can be visualized directly from the responses of biological communities in the real environment.

Our findings indicated that eRNA metabarcoding may be useful for community-level ecological risk assessment and provide important knowledge that complements the assessment results using the AF method. To properly manage chemical substances, it is important to actively incorporate the results of community-level hazard assessments as a priority and to discuss the validity of current risk assessment methods and environmental standards.

2.09.P-Tu100 Mercury Bioaccumulation in Pacific Bald Eagle Nestlings – Spatial and Dietary Differences and Influences

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We investigated how spatial and dietary variation influenced the total mercury (THg) concentrations in bald eagles (*Haliaeetus leucocephalus*) across a marine-freshwater-terrestrial habitat gradient. Blood samples were

collected from 91 nestlings (n = 64 nests), 2021 – 2023, at sites in southwest British Columbia, Canada, including the Fraser Valley, Delta, Burrard/Howe Sound, North/South Salish Sea, Southeast Vancouver Island, Thompson River, and a reference site on the west coast of Vancouver Island. Nestling plasma was analyzed for THg, stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$), and fatty acid methyl esters (FAME) to help distinguish among marine, aquatic and terrestrial diets. Isotope signatures and FAMES had a clear spatial gradient with marine focused diets more prevalent on the outer west coast with higher $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$ and $\omega\text{-3}$ levels while transitioning to more terrestrial and freshwater diets inland with lower $\delta^{13}\text{C}$ and higher $\omega\text{-6}$ levels. When averaged across sex, THg concentrations in nestling plasma were generally higher in the Thompson River and South Salish Sea with geometric mean concentrations of 1.2 ug/g dry weight (dw) and 0.54 ug/g dw, respectively, and lower in Burrard/Howe Sound and Southeast Vancouver Island with geometric mean concentrations of 0.12 ug/g dw and 0.16 ug/g dw, respectively. Linear mixed effects modelling indicated that $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ in nestlings generally increased with increasing THg concentrations in plasma ($p < 0.05$), whereas $\delta^{13}\text{C}$ decreased with increasing THg concentrations ($p < 0.05$). We will further examine those relationships, and implications for identifying Hg sources. The lowest documented toxic effect concentrations in birds occurs at a blood-equivalent THg concentration of 0.2 ug/g wet weight; eight nestlings had THg concentrations in plasma above that threshold.

2.09.P-Tu101 Mercury Exposure and the Protective Effect of Selenium in a Wintering Population of Common Razorbill (*Alca torda*) in the Southwestern Mediterranean

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Razorbills are marine birds that breed along the North Atlantic coasts, especially in Iceland, Norway, the British Isles, and the North Sea. After the breeding season, many individuals migrate southward to overwinter in more temperate regions, crossing the Strait of Gibraltar and dispersing across the western Mediterranean basin. In this study, 27 razorbills found dead along the eastern Spanish coast were collected between November 2022 and January 2024. Necropsies were performed and samples of liver, kidney, muscle, feather, and bone were collected for analysis of Hg and Se by ICP-MS. In addition, the molar ratio Se:Hg was calculated for each tissue. The influence of biological variables (sex, age, body mass, and body condition) on metal concentrations and molar balance was also assessed.

The liver had the highest mean concentration of Hg, followed by kidney and muscle. Feathers and bone showed lower concentrations. Selenium concentrations were highest in kidney tissue, followed by liver and muscle. The molar ratio Se:Hg was above 1 in most tissues, especially in kidney and bone, suggesting a potentially protective balance. However, in several individuals, the ratio was close to or below 1 in liver and muscle, indicating possible risk of Hg-induced toxicity due to low Se levels.

Statistical analysis revealed significant differences between sexes showing higher concentrations in females. Adult birds tended to have higher Se:Hg ratios in liver than juveniles.

In some individuals, Hg concentrations in liver and kidney exceeded levels associated with reproductive or physiological harm. While Se concentrations in isolation did not indicate toxicity, the Se:Hg ratio proved more informative in evaluating potential risk. The fact that several individuals had ratios < 1 in metabolically active tissues like liver supports the concern that Se reserves may not be sufficient to counterbalance Hg burden in all cases.

In conclusion, razorbills wintering in the southwestern Mediterranean show measurable exposure to mercury, with some individuals reaching toxic levels in internal organs. Selenium appears to play a protective role, but its effectiveness depends on the relative concentration of Hg. The Se:Hg molar ratio is confirmed as a useful biomarker of toxicological vulnerability. These findings support the use of *Alca torda* as a sentinel species for evaluating trace metal contamination in marine environments.

Disclaimer/Disclosure:

Thanks to "Santa Faz" Wildlife Rehabilitation Center (Generalitat Valenciana) for helping in collection of the carcasses.

2.09.P-Tu102 A Field Study Examining the Acute Effects of Copper Applied as a Spray Fungicide in Citrus Orchards to Fruit-Eating Birds and the Garden Dormouse

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The present study assessed whether copper applications in citrus orchards in Southern Europe, following Good Agricultural Practice (GAP), cause acute effects on fruit-eating birds and garden dormice. Conducted in Valencia (Spain) and Algarve (Portugal) across seven orchards (six orange orchards were selected in Spain and one in Portugal, representing the common size and basic structure of citrus orchards), treatments were applied twice at BBCH 73 (2.4 kg Cu/ha) (5-day interval in Spain, 7-day interval in Portugal) under suitable conditions in May 2024.

A total of 68 birds from seven species (blackbird, great tit, greenfinch, magpie, nightingale, Sardinian warbler, serin) were radio-tagged; 63 were successfully tracked. Telemetry checks occurred before, during, and after applications to confirm exposure and monitor health. After exposure confirmation, checks continued twice daily for at least seven days post-treatment. Additional monitoring included orchard walks, night vision, nest box inspections, and camera traps.

Results showed 84% of tracked birds remained active and healthy until seven days after the second application, and 76% until at least ten days after. Exposure was confirmed for 50 birds and likely for all others. Bird monitoring recorded 3,277 sightings of 45 species, with blackbird and Sardinian warbler most frequent. Foraging behaviour occurred in 37% of sightings, confirming exposure via food intake. No signs of intoxication, abnormal behaviour, or carcasses were observed.

Six garden dormice were radio-tagged in two orchards, with 17 additional captures untagged. Exposure was confirmed for all tagged individuals. Three dormice were tracked until DAT 12, two until DAT 15, and one recaptured on DAT 17; one signal was lost early due to tag malfunction. Two dormice died, but post-mortem excluded copper as cause, attributing deaths to collar issues. Visual observations (33 sightings), camera traps, and nest box checks confirmed normal behaviour and health, with no signs of sickness or copper-related effects.

Overall, based on the radio-tracking results as well as further monitoring methods, there was no evidence either of observable acute mortality or abnormal behaviour which could be directly related to the copper applications. In conclusion, there were no indications for acute effects for both birds and mammals, demonstrating acceptable risk following GAP-conform copper application in citrus orchards.

2.09.P-Tu103 Biomonitoring of Heavy Metals in Red-Billed Chough (*Pyrrhocorax Pyrrhocorax*) to Assess the Impact on Health Following the Eruption of the Tajogaite Volcano (La Palma) in 2021

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The eruption of the Tajogaite volcano on the island of La Palma in 2021 provided an opportunity to study the impact on wildlife health. A biomonitoring study was designed using an emblematic species exclusive of La Palma in the Canary Islands (Spain), the red-billed chough (*Pyrrhocorax pyrrhocorax*), as a sentinel species to assess environmental contamination and the potential risk to animal, and presumably human, health caused by the eruption. The main objective of this study was to quantify the concentration of various heavy metals in different tissues (brain, liver, muscle, bone, lung, and kidney) of choughs that died before and after the eruption, in order to estimate the potential health risks. Additionally, correlations between metals were evaluated. For the study, 18 chough carcasses were necropsied and internal tissues were collected. The samples were analyzed using ICP-MS to quantify concentrations of 14 metals. Statistical analyses were used to determine differences between pre- and post-eruption individuals. The results showed significant increases in the concentration of several metals in the studied tissues from carcasses collected after the eruption, particularly in the lungs, muscle, and liver. Metals such as Ni, Cr, Co, Cd, Fe and Al increased considerably in the tissues of the animals that died after the eruption, suggesting a rise in environmental exposure to these elements. Additionally, several significant correlations between metals were detected, indicating possible shared mechanisms of distribution and storage in the organism, or a potential common source of contamination. Overall, these findings support the hypothesis that the eruption caused an increase in environmental levels of heavy metals; however, when compared to other studies, no clearly toxic levels for birds were found in most individuals after the eruption (6). Nevertheless, this work should serve as a warning about potential ecotoxicological and health impacts on La Palma's ecosystem and its inhabitants, reinforcing the need for continued environmental monitoring of soil, water, and other sentinel species to keep animal and human health risks in the area under control.

Disclaimer/Disclosure:

Thanks to Cabildo Insular de La Palma for helping in collection of the carcasses.

2.09.P-Tu104 Assessment of the impact of metals and parasites on the cetaceans stranded along the Basque Coast (2012-2025)

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Marine mammals have a relatively long-life span, and some species are in the upper level of the food web. Thus, they can bioaccumulate the environmental contaminants in their tissues, becoming effective sentinel species for assessing the pollution of marine food webs. The marine ecosystems are continuously subjected to anthropogenic sources of contamination which can compromise marine animals' health status. Among all the pollutants present in the marine environment, special attention should be given to metals, because these are persistent, and some have the capacity to bioaccumulate and biomagnify. In the present study, the content of metals in muscle was evaluated in 7 cetacean species stranded along the Basque Coast (2012-2025). In addition, subcutaneous fat, muscle, mesentery, lungs and stomach were analysed to determine the prevalence of different species of parasites and their parasitic load. Statistical analysis was carried out to evaluate differences in metal content and correlations with different variables (sex, developmental state, corporal condition, decomposition status...) and parasitic burden. Overall, cetaceans of this study presented comparable values of metal levels in muscle to those inhabiting other localities of the Atlantic Ocean. In contrast, Ni presented high values that could compromise the health status of the dolphins inhabiting the Bay of Biscay, as well as high levels of Pb in striped dolphin and harbour porpoises. *D. delphis* showed significantly lower values than *S. coeruleoalba* ($p < 0.01$) in Hg, and *T. truncatus* ($p < 0.05$) presented lower values than the other dolphins. Parasites from the Pseudaliidae family, *Anisakis* sp. and isolated cases of trematode eggs seemed to be the most relevant parasites impacting the health of these cetaceans. Correlations between metal levels and parasitic burdens were obtained, suggesting potential synergistic effects. No significant differences were found in metal levels relative to morphometric or biological traits, nor were any significant differences detected in parasite content across species. The present work is the first report determining the levels of metals in cetaceans stranded along the Basque Coast, and their possible relationships with parasitic loads. SAREUS project is funded by Fundación Biodiversidad (MITECO, PRTR, NextGenerationEU) & Basque Government. This work was supported by the CBET+ (IT1743-22) Consolidated Research Group (Basque Government).

2.09.P-Tu105 Toxicokinetics of the UV Absorbent, UV-328, in Japanese Quail Embryos

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Ultraviolet absorbents (UVAs) are industrial chemicals used for their photoprotective properties. These emerging contaminants have become ubiquitous in various environmental media and adversely affect certain species. UVAs have been measured in colonial waterbirds and terrestrial birds from various environments. Egg injection studies from our lab demonstrated that select UVAs (UV-328, UV-329, UV-9, BP-3) were not acutely toxic to Japanese quail or Double-crested cormorant embryos. Avian livers were sampled at mid-incubation and pre-hatch. Chemical residue analysis revealed that a certain percentage of administered UVAs were found in avian embryo livers. UV-328 concentration was higher in pre-hatch quail livers compared to mid-incubation livers, suggesting accumulation. We do not have a clear picture of how UVAs are distributed across the rest of the embryo, nor do we know how much each UVA accumulates or is metabolized in the liver and/or other organs of interest. Therefore, the objective of the current study was to investigate the partitioning of UV-328 in various tissues and compartments (e.g. shell membrane, embryonic contents) in developing Japanese quail embryos. UV-328 is of international interest based on its accumulation potential, physicochemical properties and potential for hazard, warranting its addition to Annex A of the Stockholm Convention. Based on preliminary data and literature, I predict UV-328 will accumulate in the quail liver throughout incubation. An ongoing mass-balance experiment in quail embryos will allow us to determine UV-328 toxicokinetics in quail and subsequently improve our risk assessment for this relatively novel chemical.

2.09.P-Tu106 First determination of Persistent Organic Pollutants (PCBs, PAHs and BFRs) in tegument of six species of cetaceans stranded along the Basque Coast (NE Iberian Peninsula)

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Marine ecosystems are particularly vulnerable to toxic and persistent chemical compounds, as they are continuously subjected to anthropogenic sources of contamination. Furthermore, projections indicate that pollutant concentrations in marine environments will escalate in parallel with ongoing population growth and the intensification of global industrial production. Due to their position in the food web and large lipid deposits, marine mammals are exposed to the highest concentration of pollutants of all marine wildlife. Thus, they are vulnerable to the bioaccumulation, biomagnification, and lactational transfer of lipophilic and hydrophilic persistent organic pollutants (POPs). Elevated PCB concentrations are still a significant cause of European cetacean population's decline, and levels of this compound exceed all known protective thresholds in multiple dolphin species. Biomagnification and consumption of lipid-rich tissues enhance the risk of endocrine and metabolic disruption, which can result in alterations to reproduction, metabolic and immune function, and may influence populations' dynamics. In the present study, the content of POPs in tegument samples from 41 individuals belonging to six species of marine mammals stranded along the Basque Coast (2012-2023) was evaluated. The analyses focused on three main groups: polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and brominated flame-retardants, including polybrominated diphenyl ethers (PBDEs) and new flame-retardants (EHTBB, BTBPE, BEHTBP). The results showed wide variability in contaminant concentrations between species, sexes and maturity. PCBs had the highest concentrations, especially in adult males of *Stenella coeruleoalba* (SCO), with Σ7PCBs values above 70 mg/kg lipid weight. PAHs showed concentrations one or two orders of magnitude lower, with maximums in *Ziphius cavirostris* (ZCA) and *Tursiops truncatus* (TTR), while PBDEs reached higher levels in some individuals, notably in a TTR male with more than 1500 µg/kg lipid weight. Results were within the expected range for populations in the northwestern Atlantic. The present work presents the first record of POPs in cetaceans stranded along the Basque Coast. SAREUS project is funded by Fundación Biodiversidad (MITECO, PRTR, NextGenerationEU), and Basque Government. This work was supported by the CBET+ (IT1743-22) Consolidated Research Group (Basque Government).

2.09.P-Tu107 Skuas as Bioindicators of Emerging Pollutants in Antarctica

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Poly- and perfluoroalkyl substances (PFASs) are highly stable fluorinated compounds widely used in industrial and consumer products. They reach aquatic ecosystems via atmospheric and oceanic transport; persist in the environment, and bioaccumulate through the diet, leading to elevated concentrations in top predators. In organisms, PFASs bind mainly to albumin and lipoproteins in blood, and to fatty acid transport proteins in the liver. Particularly in Antarctica, atmospheric transportation, research stations activities and plastic debris presence may act as a dispersion route for PFASs. Brown and South Polar skuas (*Stercorarius antarcticus* and *S. maccormicki*) breed in Antarctica and can nest in sympatry. Both species have generalist and scavenger feeding behaviors. However, Brown Skuas diet rely primarily on land-based resources, while South Polar skuas are forced to feed on marine prey when occur in sympatry. Previously, we demonstrated spatial and interspecific differences in exposure risk to other pollutants in skuas across the Southern ocean, and that skuas are adequate bioindicators of emerging pollutants in Antarctica. This study aims to i- examine serum PFASs concentration in Brown and South Polar skuas along a latitudinal gradient (Potter Cove-South Shetlands ? Hope-Northern Antarctic Peninsula ? Cierva Cove-West Antarctic Peninsula), ii- determine serum trophic markers ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and iii-assess trophic drivers of PFASs contamination. Serum was obtained from blood samples taken from adult skuas (n=100) from three Antarctic locations during incubation. PFASs were quantified in serum using an ultra-performance liquid chromatography system coupled to tandem mass spectrometry (UPLC-

MS/MS), while the serum isotopic composition was analysed with a continuous flow-isotope ratio mass spectrometer. Differences in feeding habits were observed between species and locations. Brown skuas at Hope Bay and Potter Cove display a high reliance on land-based resources (such as penguins), whereas, South Polar skuas feed mainly on marine resources (such as fish). Higher loads of PFASs are expected to be found in skuas that breed in northern latitudes. Furthermore, PFASs loads are expected to be associated to feeding traits of each species, being higher in skuas feeding on land based resources which are more exposed to anthropogenic activities. Understanding PFAS presence in polar food webs is crucial for assessing their sources, distribution, and long-term impact.

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2.09.P-Tu108 First Assessment of PFAS Exposure in East African Lions Using Targeted Analysis and the Total Oxidizable Precursor Assay (TOPA)

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Lions (*Panthera leo*) are apex predators expected to integrate chemical exposure across soils, waters, vegetation and prey, making them powerful sentinels of ecosystem contamination in Africa. Despite this relevance, virtually nothing is known about their exposure to pollutants and, specifically, per- and polyfluoroalkyl substances (PFAS). These “forever chemicals” are globally distributed emerging contaminants whose persistence, bioaccumulation potential and toxic effects have raised increasing concern for wildlife health. Standard targeted PFAS analyses may underestimate total exposure because many PFAS occur as precursors that remain undetected unless oxidized, as demonstrated in several wildlife studies applying the Total Oxidizable Precursor Assay (TOPA) to reveal hidden PFAS burdens.

In this study, we assessed PFAS exposure in free-ranging lions inhabiting Serengeti National Park (Tanzania), generating the first PFAS dataset for an African terrestrial apex predator in the framework of the Marie Skłodowska Curie-funded project LEOTHREAT. Plasma samples collected through the project were analyzed by UPLC-MS/MS to quantify a broad suite of legacy and emerging PFAS. To evaluate the contribution of oxidizable precursors, paired aliquots of each plasma sample were subjected to TOPA, enabling direct comparison between native and oxidized PFAS profiles. This dual approach allowed us to characterize both directly measurable PFAS and those formed through precursor oxidation, providing a more complete representation of total PFAS exposure than targeted analyses alone.

The integration of targeted PFAS quantification with TOPA offered a robust framework to investigate PFAS pathways in lions and the ecosystems they inhabit. The study design also enabled the identification of potential precursor-rich sources that might contribute to PFAS accumulation through trophic transfer or environmental interfaces. By applying this two-tiered analytical strategy, this project establishes the methodological foundation necessary to explore PFAS behavior in African terrestrial food webs, a region and taxonomic group that have received minimal attention in contaminant research.

By generating the first PFAS dataset for East African lions and explicitly incorporating precursor assessment, this study provides essential baseline information for understanding PFAS dynamics in African ecosystems and supports future evaluations of contaminant exposure and wildlife health.

2.09.P-Tu109 COLLABORATING WITH AN INDIGENOUS COMMUNITY TO STUDY ARCTIC CONTAMINANTS: 25 YEARS OF RESEARCH WITH QIKIQTARJUAQ, NUNAVUT

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In 2000, we initiated work with the Inuit (Indigenous) community of Qikiqtarjuaq, Nunavut in Arctic Canada, on eastern Baffin Island, to create two new National Wildlife Areas (NWAs) at large seabird colonies. These colonies are located close to former weather and radar stations established during the Cold War, and the community was concerned about contamination of wildlife near these sites, because Inuit still rely heavily on wildlife as primary food sources. To address their concerns, we undertook studies in the mid-2000s to determine trace element and legacy persistent organic pollutants in local animals, establishing that levels were consistent with regional background concentrations, as well as collaborating on an Indigenous Knowledge study on the region. Following the establishment of the NWAs in 2010, the community and government formed the Sululiit

Area Co-Management Committee, where the community plays the major role in driving management, research, and monitoring priorities for the sites. As we continued to work with the committee, starting in 2018 they sought information and advice to address a major Strategic Environmental Assessment for the Baffin Bay marine region for future oil development, and thus we collaboratively undertook new studies with the community to address their concerns over increased shipping, increased fisheries, increased tourism, oil-related contamination, and new contaminant threats. In particular, in nearby environmental matrices we have completed or ongoing studies examining: macro- and microplastics pollution, plastic-associated chemicals (organophosphate esters, ultraviolet stabilizers), polycyclic aromatic compounds (PAC), fisheries bycatch, toxic trace elements, and wildlife health metrics. We present some key products from this research including a toxicogenomics “toxchip” to assess PAC exposure in wild birds, wildlife biovectoring of plastics and identification of matrix-specific microplastics differences, and different fuzzy cognitive maps produced by local residents and southern scientists on threats to wildlife in this region. Importantly, we identify successful steps we took to support this ongoing, mutually beneficial research and monitoring program.

2.09.P-Tu110 Understanding Spatial Patterns of PFAS Exposure for two Terrestrial Species in the UK

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Per- and polyfluoroalkyl substances (PFAS) are now unequivocally recognised as a major environmental concern, necessitating urgent policy interventions to mitigate widespread contamination and exposure. The identification of exposure patterns can be an important step to prioritise necessary policy changes for effective regulation to better protect vulnerable species and ecosystems. However, PFAS present a challenge in this regard due to the large number of compounds, associated properties and variety of potential exposure sources. National, long-term monitoring data for PFAS in wildlife provides valuable information to address this evidence gap. As part of a UK government-led chemical monitoring programme, liver residue data for 40-55 PFAS have been collected from two sentinel species in England - the common buzzard (*Buteo buteo*; n=180) and the European red fox (*Vulpes vulpes*; n=230) - across the period 2014–2024. In this study, these wildlife exposure data are spatially integrated with the EU Forever Pollution Project PFAS dataset, alongside UK-specific datasets that capture other potential PFAS emission sources. Combined with species-specific ecological parameters, such as home range and habitat use, these data will be analysed to assess whether any particular sources of PFAS contamination are influencing observed exposure patterns in the focal species or localised areas. Results will be presented for both species, highlighting associations between PFAS residues and known contamination sources, and evaluating whether any UK protected sites may be disproportionately at risk from this contaminant group. By linking wildlife monitoring with spatially explicit pollution datasets, this work will strengthen the evidence base for national-level PFAS policy development as well as the provision of localised advice to managers of protected sites in England. Ultimately, these findings aim to increase awareness and our understanding of PFAS exposure in terrestrial wildlife, and contribute to broader efforts to maintain environmental quality in the face of persistent chemical pollutants.

2.09.P-Tu111 From Guidance to Practice: Implementation Challenges and Strategic Approaches for Applying fTWA in Wildlife Risk Assessment

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Since the publication of the EFSA revised Guidance document on the risk assessment for birds and mammals in 2023 and its notification in October 2025, the concept of the time-weighted average factor (fTWA) has gained considerable attention. The fTWA accounts for pesticide residue decline over time and may be applied in long-term risk assessments for birds and mammals if relevant long-term toxicity effects (within a factor of 3 of the lowest effect value, i.e. “rule of three”) are driven by long-term exposure, not by short-term exposure during critical or sensitive life stages (e.g. primary effects on reproduction or development). Where application of the fTWA is deemed inappropriate, exposure estimates increase significantly, which is likely to require a higher-tier assessment, rendering substance-specific residue decline studies unusable and thus limiting the available options for risk refinement.

Despite the availability of the revised guidance and further recommendations from the Post Approval Issues Group, uncertainties remain regarding its practical implementation at the product (re-)authorisation stage. A primary challenge arises from the need to re-evaluate all relevant long-term avian and mammalian toxicity data to verify fTWA applicability, encompassing the EU-agreed endpoints and beyond, to comply with the “rule of three”. Such reassessment is not foreseen at the national level once EU-agreed endpoints have been established and may lead to divergent interpretations and compromise harmonisation across Member States. Furthermore,

detailed re-examination of study reports is often not feasible for applicants other than the original EU notifier, due to restricted access.

Against this background, we propose strategic approaches to navigate various likely scenarios while verifying the fTWA applicability. These approaches are intended to enable targeted data interpretation and facilitate the preparation of robust and compliant regulatory dossiers in support of the (re-)authorisation of plant protection products. Early adoption of a structured and harmonised strategy for evaluating fTWA applicability can provide distinct procedural and scientific advantages, including timely identification of data gaps and methodological uncertainties. It also promotes a more efficient alignment of data interpretation, endpoint justification and potential refinement options, thereby reducing the likelihood of additional information requests during the evaluation phase.

2.09.P-Tu112 EchoGO: A Cross-Species Consensus Framework for Functional Enrichment in Non-Model Organisms

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Functional enrichment in non-model organisms is often limited by sparse annotations and the narrow perspective of single-species analyses. EchoGO, an open and reproducible R package, addresses these constraints by integrating GSeq enrichment from experiment-specific annotations with orthology-based enrichment across multiple reference species. The framework builds on a simple premise: many biological pathways originated before major evolutionary splits. When orthologs of differentially expressed genes yield the same enriched terms across several well-annotated species, the replicated signal is more likely to reflect a true biological pattern. EchoGO operationalises this by running enrichment independently in each species and identifying terms that consistently appear across GSeq, g:Profiler, and taxa.

EchoGO provides two complementary outputs. The True Consensus stream retains terms jointly supported by GSeq and background-corrected orthology-based enrichment, offering high interpretive confidence. The Exploratory stream includes all significant orthology-derived terms for broader hypothesis development. A transparent scoring system integrates multi-species support, fold enrichment, statistical strength, and provenance, while semantic similarity reduction and network analysis group redundant terms and reveal coherent functional modules.

EchoGO is particularly valuable for de novo or poorly annotated organisms, where GSeq alone provides limited certainty. Across multiple datasets, EchoGO increased enriched GO terms from 64 (GSeq) to 345 (EchoGO), a five-fold improvement in depth and coverage. In Biological Process networks, GSeq produced 25 terms and 108 edges, whereas EchoGO yielded 123 terms and 2,876 edges, revealing functional organisation that was otherwise invisible. Because EchoGO tests enrichment independently in each reference species and tracks term provenance, additional terms reflect replicated signals rather than annotation inflation. This yields clearer ontology structures, more robust similarity clusters, and denser network topologies. Compared with tools such as Fun2Seq, AllEnricher or PipeOne-NM, EchoGO uniquely combines bias-aware enrichment, independent multi-species validation, and transparent consensus scoring rather than simply aggregating ortholog annotations.

Altogether, EchoGO provides a reproducible and biologically grounded framework that strengthens functional interpretation in ecotoxicology and environmental omics.

2.09.P-Tu113 The Atlantic Blue Crab as a Cross-Ecosystem Outlier: A Framework for Wildlife Toxicology and One Health

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The invasive Atlantic blue crab *Callinectes sapidus*, now widely established in Mediterranean coastal ecosystems, represents an ecotoxicological outlier due to its distinctive physiological traits, ecological plasticity, and complex exposure pathways. These features likely modulate contaminant absorption, distribution, metabolism, and excretion in ways that differ markedly from standard ecotoxicological model taxa. Despite its

invasive status, the species is increasingly incorporated into Mediterranean fisheries, raising concerns about its role as a vector of contaminants across lagoon and coastal marine systems.

However, toxicological and ecotoxicological data for *C. sapidus* under variable environmental conditions remain scarce. Here, we present preliminary results on behavioural, physiological, and sub-organismal responses to neonicotinoid exposure, complemented by field sampling in two semi-enclosed coastal systems with contrasting physicochemical profiles and resource availability. Analyses include bioaccumulation of trace metals, polycyclic aromatic hydrocarbons, organochlorinated and organobrominated compounds, and neonicotinoids, as well as biomarker responses across multiple tissues (biotransformation enzymes, metallothioneins, acetylcholinesterase, antioxidant enzymes, and lipid peroxidation).

These results provide baseline information on contaminant loads and biomarker profiles under differing environmental regimes and reveal pronounced sensitivity to neurotoxicants targeting crustacean cholinergic pathways. Overall, the findings support the potential of *C. sapidus* as an outlier sentinel species within integrated environmental pollution assessments, highlighting its relevance at the interface of ecological and human health risk.

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2.09.P-Tu114 Revised EFSA Wildlife GD: Field study scale matters for birds and mammals - representativeness reconsidered

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For environmental risk assessments of pesticides, e.g. movement or occurrence data of birds and mammals in the agricultural landscape can be considered. The EFSA Bird & Mammal 2023 Guidance suggests that selected landscapes used for crop-related higher tier field studies to investigate vertebrates within the landscape should preferably contain at least 33.3% of the respective crop type. This fixed proportional threshold is intended to ensure that study sites are considered representative and conservative regarding assumed crop-species relation. However, this value is actually difficult to be classified without reference to the size of the area, and even for widespread and commonly grown crops like cereals > 30% coverage is rarely achieved in wider agricultural landscapes across Europe. As a result, the threshold often proves to be unrealistic for practical site selection regarding areas needed to contain diverse structures, such as several fields of different crops alongside natural habitats, water bodies or anthropogenic structures.

Moreover, vertebrate species interact with their environment at multiple spatial scales — from very local activity to e.g. large-scale movement ranges. Using habitat models and multi-scale mapping, we illustrate how the perceived representativeness of a ‘study site’ strongly depends on the chosen spatial frame. Small changes in the defined boundary can substantially affect the calculated crop share, while the ecological situation for the species remains unchanged.

We argue that representativeness should be based on the species and its ecology rather than on a numerical threshold. That would provide a more meaningful basis for field study site selection, improving ecological relevance and revealing most realistic field data for reliable use in environmental risk assessments for pesticides.

2.09.P-Tu115 Northern fulmar and black guillemot as monitoring organisms for environmental pollutants

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Black guillemot has been used as a monitoring organism for environmental pollutants for several years as part of the AMAP environmental monitoring in the Faroe Islands. Northern fulmars have also been monitored during the same time period, however more irregularly. In recent years, it has not been possible to obtain sufficient samples of black guillemot, and the monitoring has thus focused more on the Northern fulmar. The two species have different ecology and behaviour which may influence their exposure to various environmental pollutants analysed in the monitoring programme. In addition, there is also a partial human exposure purpose of our monitoring. Although black guillemot is a protected species (since the 1950s) and have not been consumed to a great extent in the Faroe Islands in recent years, the fulmars on the other hand are a popular food, and are consumed both as eggs, fledglings and adults, so monitoring fulmars holds a special human exposure relevance in addition to their ecological role and as a sentinel species.

Contaminants such as mercury (Hg) and persistent organic pollutants (POPs) have been analysed in black guillemot eggs since 2000, and heavy metals have been analysed in black guillemot liver since 1999. Fulmar eggs have been analysed sporadically since 2000 and became the primary seabird egg matrix in the monitoring programme from 2016. Fulmar muscle, and in some cases also liver, have been analysed for metals and POPs in the late 1990s and again since 2008, with PFAS included from 2016. The contaminant levels in black guillemot and fulmar are not directly comparable: Hg concentrations are higher in black guillemot, whereas POP concentrations are substantially higher in fulmar eggs. These differences are most likely due to different foraging methods, different distribution ranges, and other biological factors such as age at sampling. Contaminant levels in black guillemot are considered more representative of the long-range pollution reaching the Faroe Islands, since the black guillemot is more sedentary, forages locally, and has a more selective diet. Fulmars, on the other hand, forage over a wide area and have an opportunistic feeding strategy, resulting in contaminant levels that reflect pollution across a much broader geographical region. However, monitoring pollution in fulmars remains relevant for assessing human exposure, since fulmars and their eggs are still used as a food source.

2.09.P-Tu116 Implications of the Updated EFSA Guidance on Birds and Mammals: Evaluating the Use of the fTWA Factor in Risk Assessment

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Risk assessment for birds and mammals exposed to plant protection products (PPPs) in the European Union has undergone a significant methodological shift with the transition from EFSA's 2009 Guidance to the updated EFSA 2023 Guidance, implemented in October 2025. Under the 2009 framework, chronic dietary exposure assessments systematically incorporated the time-weighted average factor (fTWA) to account for residue decline in treated crops over time. This approach assumed that long-term exposure was best represented by averaged concentrations, irrespective of the toxicological endpoint or residue dissipation dynamics.

The 2023 Guidance introduces a more nuanced paradigm: the application of fTWA is no longer default but contingent upon scientific justification. Specifically, its use depends on the nature of the endpoint (e.g., reproductive vs. growth) and whether chronic effects are driven by sustained exposure or short-term critical windows. This change introduces a requirement for endpoint-driven justification, ensuring that exposure estimates reflect the biological interpretation of the study results.

In this poster, we evaluated the impact of this regulatory shift by comparing risk assessments for selected active substances using publicly available ecotoxicology data used to define the regulatory agreed endpoints. For each substance, an assessment was made to evaluate whether fTWA application is justified under the new criteria and quantified its influence on toxicity-exposure ratios (TERs) and risk characterization outcomes.

Conversely, justified application of fTWA reduces conservatism without compromising ecological safety, particularly for substances with rapid dissipation and endpoints linked to prolonged exposure. These findings highlight the importance of transparent justification for fTWA use and consistent interpretation of study endpoints under EFSA's 2023 Guidance.

2.09.P-Tu117 How Appropriate is it to use Mammalian Model Deviation Ratios (MDRs) to Predict Mixture Toxicity in Birds?

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The new bird and mammal risk assessment guidance for plant protection products (PPPs) (EFSA 2023) requires mixture toxicity risk assessment using dose addition. If experimental data are available, a model deviation ratio (MDR) is calculated to estimate how well experimental data fits predicted mixture toxicity. According to EFSA (2023), when $MDR > 3$, effects greater than additive are assumed, suggesting synergistic interaction. However, MDRs are usually only calculable for acute mammal endpoints because bird formulation studies are unlikely to have been conducted (vertebrate testing reductions where possible). Therefore, extrapolation from mammals to birds is currently proposed, which assume physiological similarities and comparable sensitivity between taxa. This analysis evaluated whether extrapolating mammalian MDRs to predict mixture toxicity in birds is scientifically justified, and investigated potential sources of differences in mixture toxicity between birds and mammals.

Acute toxicity data and MDRs for 84 formulated products were analysed, comparing bird and mammal datasets. MDRs were evaluated based on mode of action, pesticide type, and formulation type. Study methodologies and physiological differences between species were examined. Toxic units (TUs) were investigated to explore

differential sensitivity patterns using a representative formulation as a case study.

All bird formulation studies with MDR > 3 (7/7, 100%) were derived from unbound endpoints (no effects at highest dose tested). Similarly, most mammalian formulation studies with MDR > 3 (7/9, 78%) were from unbound endpoints. In addition, TU analysis from the case study revealed that, although the compound with highest TU was highly toxic to birds alone, mammals showed greater sensitivity to the complete formulation, indicating different toxicity profiles across taxonomic groups.

The prevalence of unbound endpoints driving high MDRs suggests these values reflect testing limitations rather than true synergistic toxicity. Extrapolating mammalian MDRs to birds appears overly conservative due to physiological differences and data limitations, potentially inflating mixture toxicity risk assessments for birds. These findings underscore the importance of testing both taxa, as active ingredients exhibit different toxicity profiles across taxonomic groups that cannot be predicted from single-species data. New approach methodologies (NAMs) might be supportive to avoid further vertebrate testing.

2.09.P-Tu118 Bridging the Gap Between First Tier and Higher Tier Risk Assessments for Seed Treatment Plant Protection Products: Refinement Options for Birds and Mammals

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Seed treatment plant protection products (PPPs) provide protection to crops against pathogens, diseases and pests from germination. Authorisation of these PPPs is subject to demonstration that their use does not result in unacceptable effects on non-target organisms, including birds and mammals. Demonstrating that the actual protection goal has been met, by clearly establishing that there will be no visible mortality and no long-term repercussions for abundance and diversity, has often proved challenging. In the UK, this has resulted in a reliance on emergency applications or import of treated seeds to meet growers needs.

The first tier risk assessment scheme for seed treatments in the EFSA bird and mammal guidance (EFSA 2009 and 2023) uses generic focal species to assess exposure and risk based on worst case assumptions. The treated seed itself can be a food source for granivorous species and the emerged seedlings can be a food source for herbivorous species.

In order to move away from worst case assumptions, data are required to refine the risk assessment. Some of these data are substance specific, others are common across substances and relate to the ecology and behaviour of birds and mammals.

Since the introduction of EFSA (2009), data on focal species and their use of farmland have been collected by regulatory authorities and applicants for authorisation of PPPs. There is also published ecological data in the scientific literature.

The UK currently relies on applicants to bring together the information they have generated or have available to perform the risk assessment, which can require complex weight of evidence approaches. The aim of this work is to bring together existing information from a variety of sources to provide standardised refinement options. This simplifies the initial stages of a higher tier risk assessment, while ensuring the required protection goal is met.

The work has considered unprotected data available to HSE to identify suitable bird and mammal focal species for a range of seed treatments in the UK. Refinements of the proportion of diet obtained in the treated area (PT) and the composition of diet obtained within the treated area (PD) associated with those focal species have been derived, where sufficient data exists, thus providing robust refinement options accessible to all applicants.

The options presented do not prevent the use of other refinements considered in the EFSA guidance, when suitable data are available.

2.09.P-Tu119 The known unknowns – An approach how to assess adverse effects of Anticoagulant Rodenticides in wildlife

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Anticoagulant rodenticides (ARs) are found in wildlife and pets in many European countries and elsewhere. Their occurrence is primarily linked to the use of ARs as biocidal products for pest management and also to their use as plant protection products. However, little is known about effect levels, toxic thresholds, and precise routes of uptake in non-target animals. Secondary poisoning of non-target animals has been demonstrated by monitoring data in mammals (e.g. foxes, cats, dogs, pigs, sheep, bats) and birds (e.g. owls, kestrels, geese). This is assessed in regulatory dossiers, but calculations are mostly based on effect data from target animals. Toxicological data are often scarce, and when available, they frequently originate from the 1970s and 1980s and provide very little detail on data collection methods. Furthermore, more than one AR is often found in the livers of free roaming cats, dogs, or wildlife. In contrast, mixture effects are not addressed in regulatory dossiers. Here we present a collection of reviewed toxicological data from publicly available sources. These data were used to assess risks to wildlife and pets in a harmonised manner. This may help to improve understanding of the overall impact of ARs on non-target animals.

2.10 Microbial Ecotoxicology: From Ecosystem Resilience to Environmental Health

2.10.T-01 Pollutant Biodegradation Profile Mediated by Multi-trophic Microbial Dynamics in Rivers

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Microbial communities and environmental conditions are closely linked to ecosystem functions and directly govern the biodegradation of pollutants in aquatic environments. However, the role of multi-trophic interactions and their spatiotemporal dynamics in these processes remains poorly understood. Here, we examined how seasonal and spatial variations, mediated by trophic interactions within benthic microbial communities, influence their composition, functional capacity, and collective potential to degrade a diverse array of organic pollutants in rivers. By characterizing both prokaryotic (i.e., archaea and bacteria) and eukaryotic taxa (i.e., algae, fungi, protists, and metazoans), and inferring metabolic pathways, we explored the connections between community composition and pollutant degradation in wastewater-receiving rivers across four seasons. Mediation analysis revealed that multi-trophic communities mediate the total effect of environmental factors on the biodegradation of 96 organic pollutants. Prokaryotic communities explained 60% of the total environmental influence on pollutant biodegradation. Additionally, eukaryotic groups had significant indirect mediation effects, with fungal, protistan, algal, and metazoan communities responsible for 56%, 53%, 26%, and 38%, respectively. Notably, fungal and protist communities also mediated approximately 83% and 73% of the environmental impacts on prokaryotic community composition. We also found that spatial variation at the river and reach scale had a greater influence than seasonality in shaping benthic microbial communities. Our findings improve understanding of ecosystem resilience and support the development of predictive models and sustainable water management strategies in dynamic aquatic environments.

2.10.T-02 Impacts of Chronic Sodium Fluoride Exposure Followed by a Viral Challenge on Juvenile Rainbow Trout and Their Microbiomes

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In recent years, ecotoxicological research progressively adopts a holistic holobiont perspective integrating the host and its microbiomes. However, multi-stress interactions, e.g. how pollutants affect microbiomes and host disease resistance, remain largely unstudied. Sodium fluoride (NaF) is a contaminant of renewed interest found in surface waters. Its acute toxicity is described, but chronic and microbiome-mediated impacts remain largely unexplored. This study aimed to determine how NaF exposure impacts the rainbow trout (*Oncorhynchus mykiss*) holobiont, and its response to a viral challenge. Trout were chronically exposed to environmentally relevant NaF concentrations (0, 5, 17 mg/L) for 7 months, before being challenged with the Infectious Hematopoietic Necrosis Virus (IHNV). Trout tissues (gill, skin, gut) were sampled at 7 months or 96h, 1 and 6 weeks post-infection. DNA was extracted and the v3-v4 region of the 16S rRNA gene was amplified and sequenced for metabarcoding. Reads were processed using FROGS v5 and analysed for microbial diversity and composition. NaF exposure induced tissue-specific microbiome changes: while Alpha-diversity and microbial interconnexions decreased in gills, both increased in skin following NaF exposure. Beta diversity was significantly affected in the three tissues. Impacts on the microbiomes also differed depending on the exposure

concentration: Bêta diversity significantly differed between trout exposed to 5 or 17 mg/L in gills and microbial interconnexions alterations were more pronounced in 5 mg/L condition. This pattern was also observed for mortality: trout exposed to 17 mg/L exhibited higher mortality after the viral infection compared to unexposed fish, whereas this was not the case for trout exposed to 5 mg/L. An increase in the relative abundance of potential pathogenic taxa was also reported in gills and gut samples following NaF exposure. Microbiome analyses suggest a temporal evolution of microbiome diversity and composition after the infection (96 h, 7 days or 6 weeks). These analyses also enabled characterization of microbiomes from fish that survived the viral challenge. To conclude, NaF exposure significantly affected the trout microbiomes in a tissue-specific manner, and the two tested concentrations exhibited different impacts. Further analyses will explore how prior NaF exposure influences microbial communities and their relationship with host immunity after a viral challenge.

2.10.T-03 Cryogenically milled tire tread induces functional and structural changes in natural periphyton communities

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Tire and road wear particles (TRWP) are generated through abrasion and subsequently accumulated along the roadside, where they can be transported into nearby soils and surface waters. Environmental concentrations of TRWP are generally not expected to exceed 10 mg/L in streams although concentrations reaching the g/L range have been reported in extreme situations. While interest in TRWP and their related chemicals has grown, their interactions with periphytic biofilm communities remains poorly understood. Such communities are complex assemblages of microorganisms, including algae, bacteria and fungi, at the base of aquatic food webs. They have a key role in various processes, such as photosynthesis and biogeochemical cycles. Naturally growing and covering large surfaces of the freshwater streambeds, periphyton can also act as a sink for a diverse range of particles and chemicals, including TRWP. In this study, we used cryogenically milled tire tread (CMTT) as a TRWP surrogate to (Experiment 1) expose periphyton to three CMTT concentrations (50, 500, and 1000 mg/L) and a control for 14 days, and (Experiment 2) compare periphyton responses to CMTT particles (1000 mg/L) and their associated chemicals over 4 days. In both exposure scenarios, approximately 25–30% of CMTT became associated with the periphyton assemblage, highlighting its role as a sink for tire-derived particles. Photosynthetic efficiency showed slight declines at the highest concentration (1000 mg/L, Exp. 1 & 2) and in associated chemicals-only (Exp. 2) treatments, while algal biomass (chlorophyll a) decreased by ~30% after 14 days at 500–1000 mg/L. For Experiment 1, shifts in periphyton's structure and composition were observed at all CMTT concentrations tested. Above all, we observed the near disappearance of the bacterial phylum Firmicutes in presence of CMTT and substantial reductions in diatom abundance (45%, 85%, and 70% at 50, 500, and 1000 mg/L, respectively). These declines corresponded with increased relative abundances of cyanobacteria and green algae at 50-500 mg/L CMTT. In Experiment 2, exposures to CMTT-related chemicals alone produced effects comparable to those of CMTT particles, indicating that chemical leaching largely drove the observed responses. Overall, these findings suggest that periphyton communities can both influence fate, retention and effects of tire particles and associated chemicals in aquatic environments and undergo CMTT-induced ecological changes.

Disclaimer/Disclosure:

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2.10.T-04 Environmental concentrations of the fungicide tebuconazole alter microbial biodiversity and trigger biofilm-released transformation products

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Although aquatic microbes are relevant components of riverine biodiversity, they are largely neglected in current risk assessments frameworks. In this regard, ecotoxicological data on the effects of fungicides on aquatic microorganisms - including fungal communities - are essential for informed risk assessment and regulation. In the European context, azole fungicides have recently been added to the Watch List due to growing concern about their hazardous properties. In this study, we investigate the ecotoxicological effects of both chronic and acute exposure to the azole fungicide tebuconazole on the biomass and diversity of fungi, bacteria, and algae in stream biofilms using an RNA metabarcoding approach. In addition, the study uses LC-MS/MS to evaluate the capability of biofilms to metabolize tebuconazole. Natural biofilm communities from a Swedish stream were exposed chronically (24 days) and acutely (96 h) to environmental concentrations of tebuconazole (10 and 100 µg/L) in microcosms conditions. The diversity and community structure of fungi and bacteria were assessed by ITS2 and 16S cDNA metabarcoding, respectively. Biofilms chronically exposed to tebuconazole produced and released unidentified transformation products into the water column, suggesting a biotransformation capability following 24 days of uninterrupted exposure. The fungal biomass markedly decreased by a biomass loss of 40% when chronically exposed to 10 µg/L, and 60% when chronically exposed to 100 µg/L. Bacterial and algal biomass remained comparable with the controls in all tebuconazole treatments. Fungal and bacterial alpha diversity metrics were not significantly affected, although a decreasing trend in fungal richness was observed with the treatments. However, beta diversity was significantly affected in both fungal and bacterial compartments. Chronic exposures resulted in a shift in community composition, where taxa potentially more tolerant to tebuconazole (i.e. Lecanoromycetes) replaced more sensitive taxa (i.e. Malasseziomycetes). This study indicates that tebuconazole at environmental concentrations might pose a risk to freshwater systems, mainly due to its high toxicity to fungi.

2.10.P Microbial Ecotoxicology: From Ecosystem Resilience to Environmental Health

2.10.P-Tu120 Aquatic Biofilm as an Ecotoxicological Test System: A Systematic Review of Controlled Exposure Studies

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The majority of aquatic microorganisms doesn't live planktonic but is organised in biofilms covering submerged surfaces such as rocks, sand or macrophyte leafs. These complex assemblages consisting of algae, bacteria, fungi, protozoa and viruses are vital for the functioning of aquatic ecosystems, contributing to primary production, nutrient cycling, and degradation of pollutants. As ecotoxicological test system, it poses additional challenges compared to single-species assays but can offer more realistic insights into environmental effects of pollutants and allows the investigation of community level effects. Fast growth, minimal space requirements and a multitude of different endpoints make it an ideal choice for researchers. This review provides an overview of current knowledge on the effects of contaminants on aquatic biofilms, compares methodologies and highlights research gaps as a foundation for future studies.

A systematic literature search was conducted using Scopus and Web of Science to identify studies exposing aquatic biofilm to chemical pollutants under controlled laboratory conditions. Data retrieved from the study reports include biofilm origin and age, substrate, test substances, exposure design (i.e., single/mixture, duration, recovery time), endpoint categories, and whether interactions with other stressors were assessed; and analysed statistically.

Herbicides, antibiotics, and metals are the most frequently tested chemical classes investigated in the various biofilm assays. Most experiments expose aquatic biofilm to single substances; mixtures of more than two compounds are rare. Mixtures are almost exclusively composed of chemicals from the same class (e.g., two pesticides), cross-class mixtures (e.g., pesticide + pharmaceutical) are underrepresented. About half the studies test only one or two concentrations. Only few determine effect concentrations such as EC50 values, hampering comparisons. While community composition remains a common endpoint, molecular methods such as metabarcoding increasingly replace microscopy. OMICs approaches like transcriptomics and metabolomics are also emerging. Current research focuses strongly on the phototrophic community using endpoints such as chlorophyll concentration or photosynthetic activity, and to a lesser extent, bacterial metabolism. In contrast, impacts on fungi, protozoa, and viruses remain underexplored, underlining the need for more inclusive and functionally diverse testing frameworks.

2.10.P-Tu121 Optimizing a Periphytic Biofilm-based Bioassay for Ecologically Relevant Effect-Directed Analysis

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In aquatic systems, chemical contaminants usually occur as complex mixtures that can impact ecosystems even at trace levels, while standard monitoring often overlooks their combined effects. Effect-Directed Analysis (EDA) links toxicity to causative compounds by combining bioassays, chemical analysis, and fractionation. But it lacks ecological relevance as typical EDA bioassays target narrow in vitro or cellular endpoints. This study addresses these gaps by advancing an ecologically relevant EDA framework that uses natural freshwater biofilms as model communities. Made up of autotrophic and heterotrophic organisms, biofilms are sensitive to stress and serve as indicators of ecosystem condition. By integrating early metabolic disruptions with functional and community-level responses, this approach aims to identify key toxicants in mixtures and enhance the ecological relevance of EDA outcomes.

Biofilm bioassays were run in 6-L microcosms mimicking oligotrophic streams, using nutrient-enriched water, recirculation, and sterilized glass tiles for 21 days of colonization. For dose-response tests, tiles were randomly moved to 6-well plates and exposed to a nine-compound sub-mix (Diuron, Bisphenol-A, Atrazine, Terbutryn, Acetaminophen, Clarithromycin, Carbamazepine, Diclofenac, Triclocarban) spanning diverse physicochemical properties. Short-term exposures (6–48 h) at six concentrations across 0–50 µg/L included a chemical-free control and a 0.5% methanol control. Phototrophic community composition and photosynthetic efficiency were assessed at 0, 6, 24, and 48 h.

Biofilm community composition remained stable throughout the 48-hour exposure, with no significant differences across replicates or treatments, highlighting the resilience of biofilms to short-term chemical stress. In contrast, Photosynthetic efficiency was highly responsive, showing a dose-dependent drop with significant declines at 10 and 50 µg/L from 6 h through 24 h. The results show that fluvial biofilms offer sensitive, ecologically relevant endpoints suitable for integrating into Effect-Directed Analysis to identify and prioritize toxic surface-water contaminants. Upcoming work will test additional biofilm endpoints, such as enzyme activity, cell viability, live/dead bacteria ratios, and metabolomics, and validate the most suitable ones using a complex mix of 80 diverse CECs. The final goal is to apply this refined EDA to surface waters to evaluate acute mixture impacts and pinpoint key stressors

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2.10.P-Tu122 Effects of Different Types of Biochar on the Microbial Activity and Community Composition of PFAS- and PAH Contaminated Soils

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Biochar is a carbonous, porous material produced by pyrolysis of biomass, which can be potentially used as an amendment for soil remediation, whereby contaminants are stabilized and ecological restoration of soil is promoted. However, the profound effects that these sorbents can have on soil microorganisms remains an area requiring further research. Here, we investigated the effects of different biochar treatments in various contaminated soils.

Two per- and polyfluoroalkyl substances (PFAS) contaminated soils were treated with either 1% compost and

NPK fertilizer alone or combined with 2% (w/w) of either wood-based activated carbon or sewage sludge-based biochar. Two soils that were contaminated with polycyclic aromatic hydrocarbons (PAH) were treated with compost and fertilizer, alone or combined with two types of wood-based biochar, added to 3% (w/w). To analyze effects on soil microorganisms, we combined next generation sequencing providing information on microbial community structure, diversity and functional analysis, with the analysis of microbial respiration using a stable isotopic approach.

The treatments with biochar, compost and fertilizer increased the rate of microbial respiration of the soils, except for one soil that had a higher baseline soil quality compared to the other soils which were more nutrient-poor. The functional diversity of the soils decreased somewhat due to the addition of compost and fertilizer, which was also added to the treatments with biochar. However, there was an increase in the Shannon diversity index in samples treated with biochar, compared to compost and fertilizer only. The microbial community showed shifts in species composition between the treatments and some differences between the different types of biochar. A positive trend was seen in the increased presence of the bacteria *Sphingomonas* in PAH-contaminated soil, which is a known PAH degrader, potentially signaling a higher bacterial degradation capacity.

In summary, biochar and nutrient treatments have most profound effects on the microbial communities in nutrient-poor soils. The addition of biochar can be beneficial to maintain microbial diversity under nutrient addition and potentially promote contaminant degradation. A combined functional and structural approach provides better insights into the changes in microbial communities in biochar remediated contaminated soils, and can help us better assess performance and to optimize biochar remediation systems.

2.10.P-Tu123 Impacts of Perfluorobutanoic Acid (PFBA) on the Growth of Filamentous Cyanobacteria (*Microseira wollei*) and the microbial community at Environmentally Relevant Concentrations

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Per- and polyfluoroalkyl substances (PFAS) are increasingly detected in aquatic systems, yet their impacts on primary producers and associated microbial communities remain poorly understood. Perfluorobutanoic acid (PFBA), a short-chain PFAS widely found in surface waters, may pose distinct impacts on growth and community dynamics. This study investigates how PFBA exposure influences the growth of the filamentous cyanobacterium *Microseira wollei* and alters the surrounding microbial community. We conducted laboratory exposures in which cyanobacterial cultures were treated with environmentally relevant PFBA concentrations (1 ng/L, 10 ng/L, 100 mg/L, and 100 ug/L) for 21 days. Cyanobacteria were grown in all concentration with site water that was autoclaved and site water that was untouched. Cyanobacteria grown in site water that was not autoclaved at 100 ug/L grew significantly longer ($p = 0.012$) than the control group. Cyanobacteria that were grown in autoclaved site water showed no significant growth changes. Additionally, for all concentrations of cyanobacteria that were grown in unautoclaved site water, the cyanobacteria that survived for 21 days had symbiotic growth. At day 21 of the study, all the dead cyanobacteria had no symbiotic growth. Our results suggest that PFBA stimulates cyanobacteria growth and impacts the microbial community associated with this cyanobacteria species. These findings provide new insight into how short-chain PFAS may influence cyanobacterial dynamics and the structure of freshwater microbial communities.

2.10.P-Tu124 Microbial Infallibility to Degrade Per- and Polyfluoroalkyl Substances

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The microbial infallibility hypothesis suggests that microorganisms can evolve to degrade any organic compound. Per- and polyfluoroalkyl substances (PFAS) challenge the microbial infallibility hypothesis due to their extreme persistence and the exceptional stability of the C-F bonds. Despite this, emerging evidence suggests that long-term microbial exposure can induce adaptive shifts in genomic, transcriptomic, and metabolic pathways with potential relevance to PFAS transformation. In this work, we investigate how PFAS influences the bacterial genomic potential and quantify how PFAS exposure reshapes bacterial communities.

We hypothesize that: (i) long term exposure accumulates PFAS degraders, in line with microbial infallibility, (ii) short-term exposure provides insight into the genetic machinery of responders, and (iii) isolates from sites with legacy exposure respond differently to acute PFAS exposure compared to isolates from non-contaminated sites.

We collected data from controlled studies analyzing the microbiome with PFAS exposure. We assessed the

effect of short-term and long-term PFAS exposure on bacterial community composition. The meta-analysis provides a general and robust overview of microbiome shifts under legacy PFAS exposure. *Delftia acidovorans*, a taxon described as PFAS-degrading in the literature, emerged in our analysis together with several additional candidate species. These taxa will be isolated and subjected to targeted degradation assays in follow-up laboratory work. Our findings reveal candidate species for PFAS degradation and pave the way to identify the genetic background for potential PFAS remediation.

2.10.P-Tu125 Inhibition and Metabolic Responses of Aquatic Hyphomycetes to the Fungicide Tebuconazole - an Easy to Apply Laboratory Approach

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Aquatic hyphomycetes (AH) are key drivers in the decomposition of organic matter such as leaf litter in streams. Thus, AH provide an important bottom-up function for freshwater ecosystems. Although there are several studies about the effects of plant protection products on leaf associated AH communities, the sensitivities of single AH species towards fungicide stress remain understudied. In the present study, we tested the effects of different concentrations of the azole fungicide tebuconazole (TBZ) on the growth of nine aquatic hyphomycete species in 2% malt extract broth (endpoint: dry mass). Two species (*Neonectria lugdunensis* and *Flagellospora curvula*) showed significantly lower growth beginning with nominal concentrations of 50 µg/L TBZ, whereas three species (*Tetracladium marchalianum*, *Clavariopsis aquatica*, and *Anguillospora crassa*) showed no significant growth inhibition. These results confirm different tolerances of certain AH species towards fungicide stress that were reported in previous studies with leaf litter associated fungal communities. Interestingly, varying TBZ concentrations and metabolites in the malt extract broth were observed at the end of the experiments, indicating that the fungi are dealing differently with fungicide stress. This finding is supported by first results from the quantification of ergosterol (fungal cell wall molecule whose biosynthesis is inhibited by azole fungicides) as well as differing concentrations of selected TBZ metabolites in the culture medium. Further research should focus on the reasons for different sensitivities (e.g. fungicide resistance and metabolization) in order to facilitate the choice of responsive indicator organisms and the development of standard test systems for aquatic fungi.

2.10.P-Tu126 Microbial–Plant Biostimulants Enhance *Medicago sativa* Growth Under Metal and Drought Stress

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Organic agriculture has emerged as a crucial strategy for promoting sustainable and environmentally responsible food production. Microbial biostimulants (mPB) have become natural alternatives to fertilisers, stimulating plant growth by improving soil nutrient availability. Nonetheless, their performance under anthropogenic stress and water-deficient conditions remains insufficiently characterised, highlighting the need for further empirical investigation. Therefore, this study aimed to assess the performance of a commercial mPB containing *Pseudomonas fluorescens* and *Azospirillum brasilense* in a soil historically contaminated with metal(loid)s under drought stress. A plant pot experiment was conducted using two soils: metal-contaminated soil from Estarreja (Aveiro, Portugal) and uncontaminated natural standard soil (Lufa 2.2). Each soil underwent two moisture treatments: one simulating a standard condition (50% water-holding capacity, WHC) and the other simulating a drought condition (25% WHC), both with and without mPB application. The mPB was applied 5 days after seed addition to the soil using a recommended field concentration. After 21 days of growth under controlled light and temperature, soil and plant samples were analysed for germination, plant biomass, mercury concentration in dry shoot, soil enzymatic activities, pH, and electrical conductivity (EC). Results revealed that *M. sativa* germination and growth were significantly influenced by soil type, drought stress, and mPB application. Drought conditions significantly delayed germination (delay of 5 days) and reduced root length and biomass in Estarreja soil. Nonetheless, the mPB application partly mitigated these adverse effects, improving the germination index and stimulating root and shoot growth under metal stress and limited water availability. Mercury concentrations in shoots were primarily influenced by soil type, being up to ten times higher in Estarreja soil than in Lufa 2.2, regardless of mPB presence. Soil enzymatic activities (urease, β -glucosidase, and dehydrogenase) were generally reduced in Estarreja soil. Generally, drought reduced these activities, but the application of mPB enhanced dehydrogenase. Ultimately, these findings suggest that microbial inoculants can mitigate drought-induced stress and enhance plant and soil functioning in metal-polluted environments. The use

of mPB represents a promising strategy for improving crop performance under combined environmental stressors.

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2.10.P-Tu127 Alginate-bead delivery of fungal inoculum improves plant physiological response under saline stress

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Soil rot fungi can greatly impact soil structure and functionality, which may be particularly relevant for poorly structured and/or nutrient deficient soils. Fungal bioencapsulation, by providing more favorable conditions for early fungal development, may be an efficient way to introduce fungi into soil, especially in those under some type of stress, like salinity.

In the present work, lyophilized mycelium of *Pleurotus sajor-caju* was encapsulated in alginate beads produced with two different sizes (50 or 30 µL per droplet) and two viscosities (high and low, H and L, respectively). Aiming at assessing improvements after fungal beads addition, 14-days seedling emergence and growth tests were performed with *Zea mays* (maize) and *Brassica rapa* (turnip) following OECD Test No. 208 in OECD artificial soil, in the absence and presence of an abiotic stressor (salinity). The following treatments were tested: Control (no beads), 30 LV, 30 HV, 50 LV, 50 HV under no salinity (0 mS/cm) and under salinity (4 mS/cm). Plant response was evaluated through measurements of growth (length and weight), soluble sugar content, and chlorophyll content.

Results show that for *Z. mays*, salinity caused a significant increase in chlorophyll b content. Salinity also caused a decrease in the aerial dry weight in the 50 µL beads of both viscosities. There was a significant increase in sugars concentrations for the 50 LV beads compared to the control for both salinity levels. There was also a general tendency for increased production of photosynthetic pigments and weight in treatments with beads, indicating possible positive effects attributable to the beads.

For *Brassica rapa*, salinity led to a significant decline in dry weight in the controls. Salinity led to a significant decrease in chlorophyll a in control and the 50 HV beads. Results also show a tendency for higher weights in treatments with beads, which is indicative of seemingly positive effects of the bead's presence.

Overall, these results support the use of alginate beads as a vehicle to inoculate fungi into soil. Results also show a positive impact of the fungal-loaded beads in some plant growth parameters and notably, a positive impact on the ability of plants coping with salinity.

2.10.P-Tu128 Effects of the Antimicrobial Benzalkonium Chloride on the Host Microbiome of Aquatic Insects and Riparian Spiders

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Benzalkonium chloride (BAC) is a potent antimicrobial commonly used in disinfectants and sanitizers. Its use has increased globally in industrial, clinical, and household settings and it has been detected in surface waters downstream of municipal wastewater treatment plants. A whole lake experiment is being done at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) in northwestern Ontario, Canada, to assess the effects of BACs on a lake's food web. Baseline sampling was done in 2024 and BAC additions (target 2,000 ng/L) occurred weekly from June to October, 2025. As part of this larger study, we collected macroinvertebrate larvae and adults (dragonflies, damselflies, chironomids, mayflies) and their riparian spider predators (tetragnathids and araneids) before and during BAC additions from the experimental and reference lakes. Bacterial DNA was extracted from whole invertebrates (n=6-10/taxon), and the communities were characterized using 16S rRNA gene amplicon sequencing. Baseline data from 2024 indicates that larval insects had the highest diversity of ASVs whereas spiders had the lowest diversity across both lakes.

Multivariate analyses indicated that the bacterial communities of larval insects differed among taxa and lakes, and were mainly dominated by the phylum Pseudomonadota (except tetragnathids and chironomids). In contrast, tetragnathid spiders and chironomids were dominated by the phylum Bacillota. Endosymbionts such as *Spiroplasma*, *Wolbachia*, and *Tyzzzeria* were found in some larval insects and all spiders. Analyses of samples from 2025 are ongoing and will be used to assess whether BAC exposure shifts the composition of invertebrate microbiomes in this lake.

2.10.P-Tu129 Diversity of Microbial and Macroinvertebrate Communities Growing on Plastics and Bioplastics in a Pristine Mountain Stream

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Activities such as hiking, climbing, skiing and camping put significant pressure on mountainous aquatic ecosystems. Although the ecological transition encourages the use of bioplastics, it is unclear whether these materials allow colonisation and degradation by microorganisms and macroinvertebrates similar to that of natural substrata. This study compares the colonisation of two bioplastics (polyhydroxyalkanoate (PHA) and polylactic acid (PLA)), two plastics (polyethylene (PE) and polypropylene (PP)) and wood by microorganisms and macroinvertebrates in the aquatic and terrestrial habitats of a mountain stream in the Pyrenees. Each material was placed in three evenly distributed transects in the stream and riparian forest for one year. Four samples were collected, one in each season, and the substrata were analysed for bacterial and fungal diversity using MiSeq 16S rRNA and ITS3-4 gene analyses, as well as for taxonomic identification of macroinvertebrates. The results showed that the type of substrata affected the richness of bacterial species, but not their Shannon index or the richness and indexes of fungal and/or macroinvertebrate communities. Specifically, bacterial species richness was significantly higher in wood than in the plastics deployed in the stream. Non-metric multidimensional scaling plots showed that the bacterial and fungal community compositions in wood differed significantly from those on plastics in the stream. Co-occurrence networks including bacterial, fungal and macroinvertebrate taxon abundances show a greater number of nodes establishing strong correlations (Spearman's rho >0.9) in bioplastics (PLA =121, PHA =101) than in plastics (PE =96, PP =94). In conclusion, both bioplastics and plastics are colonised by communities of microorganisms and macroinvertebrates that are similar in terms of alpha- and beta-diversity, but differ in terms of community interactions. Most of the colonisation differences observed between plastic and wood substrata in the aquatic habitat were no longer apparent in the terrestrial habitat. This is probably due to greater leaching of plastic additives into the water, which would exert greater selection pressure on colonising biological species.

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2.10.P-Tu130 Tiny Tenants, Big Impact: Deciphering the Mechanisms Linking Gut Microbes to Invertebrate Physiology

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Host-associated microbiomes play crucial roles in nutrient uptake and processing, yet disturbances can profoundly affect host health. While such effects are well known in humans and mammals, consequences for (aquatic) invertebrates remain poorly understood. Evidence suggests that chemical stress can alter the gut microbiomes of invertebrates, potentially influencing host physiology. To explore these interactions, we conducted experiments with the leaf-shredding amphipod *Gammarus fossarum*, exposing specimens to the antibiotic ciprofloxacin, the fungicide azoxystrobin, and their mixture via water, food, or both, to assess direct microbiome effects. A second experiment involved exposure to the insecticide thiacloprid via water to

investigate host-mediated, indirect microbiome responses.

Exposure to ciprofloxacin, azoxystrobin, and their mixture altered the relative abundance of several bacterial classes, including Verrucomicrobiae and multiple Proteobacteria, with effect directions depending on the antimicrobial. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analyses predicted enhanced lipid and energy metabolism, particularly under dietary and combined exposure, suggesting potential implications for host physiology. Previous studies on the same species reported up to 25% higher biomass gain under these exposure routes, while waterborne exposure showed negligible effects. Although microbiome and proteome analyses from the second experiment using thiacloprid as a stressor are ongoing, we observed a dose-dependent reduction in feeding rate and significant shifts in fatty acid content and composition. These findings indicate altered lipid and energy metabolism, potentially driven by gut microbiome changes.

Overall, our results highlight the gut microbiome as a promising driver explaining physiological responses in invertebrates under chemical stress, emphasizing its relevance for understanding contaminant effects on aquatic ecosystem health.

2.10.P-Tu131 Antiviral Effects on Methane Formation in Freshwater Sediments

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Antiviral substances are used in large quantities to combat viral diseases but inadvertently enter aquatic ecosystems. Yet their impacts on sedimentary microbiomes and key biogeochemical processes remain poorly understood. Methane formation (i.e., methanogenesis) is a central anaerobic pathway in freshwater sediments fueling both organic matter processing and the release of this greenhouse gas. Considering recent evidence for other pharmaceuticals, such as antibiotics, influencing this process through alterations in the sediment-associated microbial community, we hypothesize that antiviral substances may cause a similar outcome by modulating viral-bacterial interactions.

To address this question, we conducted controlled sediment microcosm incubations and quantified methane production at six levels of the antiviral agent Oseltamivir carboxylate (0.01-1000 µg/L). In parallel, sequencing of the 16S rRNA gene was used to resolve changes in bacterial and archaeal taxa. This approach allowed us to link functional responses in methane production with community-level shifts.

Oseltamivir exposure consistently suppressed methane formation during the initial phase of the incubation. While this pattern continued throughout the study at the highest treatment, effects diminished at lower concentrations as incubation progressed. Metabarcoding revealed rather minor effects on community composition compared to earlier studies with antibiotics, which suggests a latent but ecologically meaningful disturbance.

Our findings show that antiviral agents can disrupt the activity of methanogenic communities in freshwater sediments, while structural changes are comparatively minor. This highlights a new and distinct pathway of toxic action that is rather driven by viral-bacterial interactions and their effects on bacterial performance instead of direct bactericidal effects shifting the community. This work advances understanding of how emerging contaminants influence carbon-cycling and underscores the importance of the understudied group of antiviral agents.

2.10.P-Tu132 Urban Infrastructure Impacts on Freshwater Periphyton Microbiomes: The Aachen Case Study

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Urban freshwater systems are increasingly affected by anthropogenic pressures, including wastewater treatment plant (WWTP) discharges, eutrophication, and hydromorphological modifications such as canalisation. These pressures can alter the composition of aquatic microbiota, which plays a central role in biogeochemical cycling by mediating nutrient turnover, organic matter degradation, and pollutants transformation or removal.

Periphyton, a complex biofilm assemblage colonizing submerged surfaces, is a key component of stream ecosystems, acting as a “solar-powered biochemical reactor.” Its high ecological relevance and sensitivity make periphyton a suitable bioindicator for assessing water quality and ecosystem integrity in human-impacted rivers. This study aimed to evaluate how bacterial periphyton diversity responds to urbanization and WWTP inputs along a pollution gradient in small- to medium-sized urban areas. Genomic DNA was extracted from periphyton

biofilms collected at 7 sites (i.e., reference, urban, upstream-WWTP, and downstream-WWTP). The 16S rRNA gene (V3–V4) was amplified and sequenced using high-throughput Illumina technology. Bioinformatic analyses (QIIME2, DADA2) were applied to characterize α - and β -diversity patterns and taxonomic restructuring. The river microbiome displayed a clear spatial pattern primarily shaped by urban pressure. Urban sites exhibited high diversity metrics and a markedly distinct community structure in comparison to reference conditions. Compositionally, the reference site hosted an even Proteobacteria-dominated community with Moraxellaceae prevailing in this habitat. Conversely, sites located downstream of WWTPs and/or highly impacted by urbanization were characterized by a pronounced decline of Moraxellaceae in favour of tolerant families such as Sphingomonadaceae and Comamonadaceae. Strong spatial differentiation was observed in highly impacted sites, with urban areas forming the most distinct cluster. WWTP-related locations showed moderate shifts in community composition relative to the reference site, indicating a progressive alteration consistent with the upstream-downstream gradient.

These findings highlight the strong influence of urbanization on freshwater periphyton structure and support the use of microbial indicators for assessing ecological status and monitoring human-driven impacts in freshwater ecosystems.

2.10.P-Tu133 Urban Contaminants Affect Microbe Functioning in Freshwater Streams

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In urban landscapes, wastewater containing chemicals such as pharmaceuticals and biocides is a major source of freshwater contamination. Although treated by wastewater treatment plants (WWTPs), some contaminants persist during conventional treatment steps, jeopardizing freshwater organisms and the ecosystem processes they perform. One vital process threatened by urban contaminants is leaf litter decomposition, a microorganism-mediated function essential for nutrient cycling, energy flow, and ecosystem productivity. The present study aims to quantify the impact of urban wastewater on leaf litter decomposition and to identify the structural changes in the involved microbial communities. Therefore, nine streams within the upper Rhine valley receiving WWTP effluents were studied in January 2025. To quantify the microbial decomposition rate of leaf litter, fine-mesh bags filled with leaves of black alder or beech were deployed upstream and downstream of WWTPs (n=9) for three weeks. We used different plant species as these differ in terms of chemical composition, leading to variation in microbial colonization and degradation patterns. In addition, physico-chemical properties and the pollution load of the streams were quantified. To assess the changes in microbial communities, metabarcoding analyses were performed targeting the fungal ITS2 rDNA region. We observed that alder leaf litter decomposes at faster rates than beech regardless of the position in the stream. This may be linked to the quality of alder leaf litter, which provides more nutrition for microorganisms involved in the decomposition. Additionally, decomposition rates were negatively affected by the effluents of the WWTPs. The decomposition of leaf litter was up to 20% slower downstream of the WWTPs. The chemical analysis revealed the presence of more contaminants in the downstream sites compared to the upstream site explaining the inhibition of the decomposition. The portfolio of contaminants found included Pharmaceuticals and personal care products, detergents and pesticides which might present antimicrobial effects that could lead to the reduction of decomposition. As the ecosystem function was affected by the wastewater effluents, we hypothesize that the microbial community composition also shifted to adapt to more contaminated conditions reigning downstream of the WWTPs. The metabarcoding data is currently being processed to prove this hypothesis, and should be available for presentation.

2.10.P-Tu134 Aquatic Bacterial-Fungal Interactions Modulate the Effects of the Biopesticide Kasugamycin on Leaf-Associated Microorganisms

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Pesticides sprayed on crops can reach adjacent aquatic ecosystems where they can affect various non-target organisms even at low concentrations. In response, biopesticides appear to be a more sustainable alternative because they are supposed to be more specific and more biodegradable in the environment than synthetic

pesticides. However, their effects on non-target microorganisms remain poorly documented. This study evaluates the effects of kasugamycin, a biopesticide with dual antifungal and antibacterial action, on a simplified synthetic consortium consisting of the fungus *Paraconiothyrium variabile* and the bacterium *Shewanella putrefaciens*, both of which were isolated from alder leaf litter submerged in a pond ecosystem. Microorganisms were exposed for 28 days to two concentrations of kasugamycin (1 and 100 mg L⁻¹), under mono- and co-culture conditions. Our study is particularly innovative because it integrates microbial interactions into the assessment of biopesticide effects. Bacterial and fungal biomass, a range of functional measurements (i.e., extracellular enzyme activities, respiration, litter mass loss) and kasugamycin tolerance acquisition were measured. Our results show that high kasugamycin concentration (100 mg L⁻¹) has an inhibitory effect on fungal biomass but not on bacterial biomass in monocultures. In co-culture, competition between the fungus and the bacterium accentuates the inhibitory effect of kasugamycin on fungal biomass (day 14) in parallel with a decrease in respiration rate (day 21). A greater resistance to kasugamycin, proportional to the concentration of biopesticide prior to exposure, was observed in the bacterium-fungus consortium, as attested by the higher EC₅₀ value. Kasugamycin, being highly soluble and weakly adsorbed onto organic matter, can easily reach aquatic ecosystems and impact leaf-associated microorganisms. Ecotoxicological responses differ between monoculture and co-culture exposures, with competition between bacteria and fungi exacerbating the fungicidal effect of the molecule. These results highlight the importance of integrating biotic interactions when assessing the ecotoxicological impacts of biopesticides in aquatic environments.

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2.10.P-Tu135 Microbial metamorphosis under environmental stress: restructuring of insect-associated microbiomes across life stages in a wastewater-impacted system

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Aquatic insects host microbiomes essential for digestion, immunity, and ecosystem interactions, which are strongly reshaped during metamorphosis, the developmental transition from aquatic larvae to terrestrial adults. Wastewater effluent introduces microbes, nutrients, and contaminants that may alter these microbial communities, but it is unclear whether such changes persist across development or transfer to terrestrial predators. We addressed this question by examining microbiomes of larval and adult aquatic insects (Hydropsychidae, Chironomidae, Heptageniidae), and riparian spiders (Araneidae, Tetragnathidae) collected up- and downstream of four wastewater treatment plants along the Grand River, Ontario, Canada. Whole-body bacterial communities were characterized using 16S rRNA gene amplicon sequencing, and community differences were assessed with PERMANOVA, beta dispersion, and differential abundance analyses.

Microbial composition differed strongly between life stages. Larvae harbored microbiomes dominated by anaerobic and gut-associated taxa, whereas adults and riparian spiders were dominated by more aerobic taxa. These differences may reflect the presence of an active gut in larvae, which supports gut-specialized microorganisms, in contrast to adults, which have a reduced or inactive gut, and spiders, which feed externally. Wastewater exposure influenced aquatic insect microbiomes, including enrichment of environmental and wastewater-associated taxa and reductions in core endosymbionts such as *Wolbachia* and *Rickettsiella*. Notably, reductions in endosymbionts were also observed in adults, suggesting that wastewater exposure can disrupt microbial persistence across metamorphosis and potentially impair symbiont transfer between life stages. In contrast, spider microbiomes showed no significant upstream-downstream differences, indicating limited propagation of wastewater-induced microbial changes into terrestrial predators.

Overall, metamorphosis was the dominant driver of microbiome restructuring, while wastewater effluent introduced additional, taxon and life stage specific shifts. These findings highlight how development and anthropogenic contamination jointly shape host-associated microbial communities, emphasizing the potential for wastewater to alter microbial stability, host-symbiont interactions, and cross-ecosystem microbial transfer, with implications for both ecological function and the health of aquatic-terrestrial food webs.

2.10.P-Tu136 Microbial Response Patterns as Predictors of Sediment Contamination Levels in a Korean Coastal (Gyeonggi) Bay

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Marine sediments record both natural ecosystem dynamics and human-derived contaminants. Microbial communities inhabiting these sediments react quickly to environmental pressures, and shifts in their composition can serve as sensitive indicators of sediment condition. Although associations between pollutants and microbial assemblages have been widely documented, relatively few studies have integrated these biological signals with chemical and ecological assessment frameworks to identify robust indicator taxa. In this study, we examined whether selected microbial taxa could function as consistent indicators of sediment contamination in Gyeonggi Bay, including sites designated as Special Management Zones. High-throughput sequencing revealed microbial groups that showed consistent patterns along contamination gradients. Several taxa exhibited strong concordance with both chemical (SQG-Q) and ecological (microgAMBI) indices, supporting their potential roles as tolerance- or sensitivity-based indicators. Notably, *Romboutsia* dominated at heavily impacted sites, whereas *Woeseia* was more characteristic of less disturbed sediments. Overall, these findings highlight the utility of microbial indicators as rapid, cost-effective, and ecologically meaningful tools that complement traditional chemical analyses and enhance sediment monitoring and management strategies in coastal environments.

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2.10.P-Tu137 Microplastics alters the functional response of the Protozoa *Tetrahymena pyriformis* Ekoja-Smah Faith¹, Julia Reiss², Daniel Perkins² and Anne Robertson³, (1)University of Roehampton, London, United Kingdom, (2)Centre for Pollution Research and Policy, Brunel University of London, United Kingdom, (3)Roehampton University, United Kingdom

Microplastics (MPs) are ubiquitous in the environment and contaminate aquatic systems, posing a threat to aquatic organisms at different trophic levels. The impacts of MP contamination on microzoans (microscopic animals like Protozoa), which are major drivers of the microbial ecosystems, are not fully understood. To understand the effects of MPs on microzoans, we conducted an MP exposure experiment using the protozoan *Tetrahymena pyriformis*, a model bacteria grazer in freshwater microbial systems. Six different combinations of bacteria and MP concentrations were used to determine how MP exposure alters *T. pyriformis* feeding over the course of three hours of exposure. We expected that exposure to MP would reduce *T. pyriformis* consumption of their natural prey, with increasingly higher concentrations of MP resulting in a substantial decline in ingestion rate. This will alter the functional response pattern of the consumer.

Our findings show that MP exposure can disrupt the trophic interaction of microbial communities even at low concentrations. This highlights an ecotoxicological disruption introduced by the physical presence of MP particles rather than chemical toxicity, a disruption that is not currently incorporated into the framework of environmental risk assessment. Since microzoans regulate the abundance of bacteria, nutrients, and the transfer of energy in aquatic systems, alteration in the grazing efficiency by the presence of MPs may result in a shift in community structure and obstruct normal ecosystem functioning. This study highlights the need to include microbial predators in hazard evaluation for MP and advances the mechanistic knowledge of how particulate pollutants change ecological processes at the base of the aquatic food webs.

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2.10.P-Tu138 Single-cell Analysis of Silver Nanoparticles Effects on Natural Phytoplankton Assemblages in Two Pre-alpine Lakes with Contrasting Trophic Status

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Silver nanoparticles (AgNPs) have found wide application in various commercial, medical and industrial products due to their unique properties. Their broad use has led to an increased release into aquatic environments, posing a threat to primary producers. Despite extensive research on nanoparticle toxicity, most studies have focused on laboratory cultures, leaving a critical gap in understanding their impacts under environmentally relevant conditions. To accurately assess the ecological risks of AgNPs and predict their long-term environmental impacts, it is crucial to understand how these nanoparticles interact with natural

phytoplankton communities across diverse trophic and physicochemical conditions. By integrating flow cytometry, pulse-amplitude-modulated fluorescence, optical microscopy, and AgNPs characterisation, our work links AgNPs behaviour in natural waters to taxon-specific and size-dependent physiological responses within complex phytoplankton assemblages. Our findings demonstrate that lake trophic status and community composition jointly shape AgNPs toxicity, with assemblages from the eutrophic lake being more sensitive than those from the mesotrophic one. At the single-cell level, cyanobacteria and smaller phytoplankton species emerged as the most vulnerable groups, highlighting fundamental ecological traits driving nanoparticle effects in situ. This study provides novel insights into the impacts of AgNPs on freshwater ecosystems by demonstrating that differences in water chemistry and phytoplankton community composition between eutrophic and mesotrophic lakes play a determinant role in shaping AgNPs effects. Furthermore, this study emphasises the value of high-resolution single cell techniques for evaluating the impact of anthropogenic pollutants on natural phytoplankton assemblages.

2.10.P-Tu139 Fitness Cost and Community Stability Shape Multidrug-Resistant Bacterial invasion in Freshwater Sediment

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The spread of antibiotic resistance is a worldwide health, economic, and environmental concern, particularly in hotspots like wastewater treatment plants (WWTPs), which release antibiotic residues and antibiotic-resistant bacteria (ARB) into freshwater environments, possibly contributing to the spread of antibiotic-resistant genes (ARGs). However, the success of ARB in natural communities depends on the fitness of the invader and on the biotic resistance of the receiving community. This study examines the fitness cost of bacteria carrying MDR plasmids and their invasion capacity in freshwater sediment. Moreover, we tested whether low antibiotic levels can disrupt sediment microbial community structure, increasing ARB invasion potential and ARGs spread. We assessed plasmid-associated fitness costs through growth assays in freshwater, comparing a plasmid-free *Escherichia coli* CV601 strain with an MDR transconjugant. To determine whether low antibiotic concentrations could alter sediment microbial community structure and promote ARB invasion and/or ARG dissemination, we conducted a 10-day static microcosm experiment with five treatments: (i) control, (ii) antibiotic mixture (trimethoprim 10 µg/L, cefotaxime 4 µg/L, ciprofloxacin 1 µg/L), (iii) *E. coli*, (iv) MDR *E. coli*, and (v) MDR *E. coli* with antibiotics. MDR *E. coli* exhibited significantly reduced growth in freshwater, indicating a fitness cost of plasmid carriage. In sediment, the MDR strain showed limited capability to invade sediments compared to a plasmid-free strain, and ARGs associated with the plasmid were detected only one day before their decline. Sediment bacterial diversity and community composition remained stable across treatments, suggesting that low antibiotic concentrations in water did not disrupt sediment community structure. Together, the community's stability and the fitness burden of the plasmid likely constrained the establishment of MDR *E. coli* and the spread of ARGs. Overall, our findings indicate that MDR plasmid carriage restricts bacterial invasion in freshwater sediments, and that sub-inhibitory antibiotic levels in water do not necessarily facilitate invasion in freshwater sediments. Nevertheless, the transient detection of ARGs despite low invader establishment suggests that continuous WWTP inputs may still contribute to the persistence of resistance genes in freshwater ecosystems.

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2.11 Non-Target Arthropod Risk Assessment: A Simple Matter of Protection Goals or a Complex Web of Interactions?

2.11.T-01 Protocol for the revision of the risk assessment of terrestrial non-target organisms

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The protocol for the revision of the EU terrestrial guidance document for the risk assessment of pesticides, covering non-target arthropods (NTAs), in-soil organisms, and non-target terrestrial plants (NTTPs), falls within the remit of the EFSA mandate M-2024-00086. It describes the problem formulation, evidence needs, and approach for Tier 1 RA developed by the Environmental Risk Assessment Working Group (ERA Working Group) and the EFSA Panel on Plant Protection Products and their Residues (PPR Panel). The protocol follows the principles outlined in the EFSA Guidance on protocol development for EFSA generic scientific assessments. It was considered beneficial for the Tier 1 risk assessment of the three GD since it allows for a transparent and balanced approach for the revision of the guidance document. From a biological standpoint, the compartmentalisation of the terrestrial ecosystem for regulatory risk assessment is recognised as arbitrary. However, the regulatory framework simplifies the inherent complexity of natural systems through this separation, since addressing the risk of PPPs to all organism groups within a single guidance document would be extremely complex. Nevertheless, the combined protocol allows for efficiency and harmonisation of cross-cutting parameters and approaches.

The protocol defines the evidence needs and approaches for addressing data gaps, thereby establishing the basis for the forthcoming revision of the three Guidance Documents. The protocol is composed of an introductory part followed by a section specific to NTAs, in-soil organisms and NTTPs. To ensure a harmonised approach across the three protocols, the concepts of source of emission, exposure pathway, exposure-relevant environmental matrix, and exposure route were introduced in a common introductory section.

Gaps in the current risk assessment of NTAs, in-soil organisms, and NTTPs were identified initially based on the EFSA PPR Panel Opinions [3,4,5] and other available documents, such as the report from the 2025 workshop co-organised by EFSA and the European Commission [6] and were further complemented with expert judgement. Each identified gap was assigned a relative priority score using expert judgment and the appropriate method for collecting additional evidence was selected.

The presentation will focus on the main highlights of the protocol and the comments received during the public consultation that should run from December 2025 until end of January 2026.

2.11.T-02 Moving forward on Non-Target Arthropods Environmental Risk Assessment - Are Current Tier 1 Species Protective Enough?

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European environmental risk assessment of plant protection products includes an assessment of risks to terrestrial non-target arthropods (NTAs). As a data requirement in the first Tier, effect studies with two 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 is sufficiently protective of vulnerable key driver NTA species in the field. By generating comparable sensitivity data across a broader range of functionally relevant species, this work provides a valuable evidence base for evaluating how protective the current “surrogate” species are. For that purpose, cultured NTA species from 16 representative arthropod families were exposed in dose-effect series to three formulations of insecticides with different modes of action - lambda-cyhalothrin, flupyradifurone and cyantraniliprole - via overspray application. Effects were assessed 4h, 24h and, at least, 48h after exposure. Medium Lethal Doses (LD50) were derived and grouped per insecticide in Species Sensitivity Distribution (SSD) curves. Across insecticides, the most sensitive species overall were parasitoids such as *Encarsia formosa*, *Trichogramma achaea* and *A. rhopalosiphii*, while the least sensitive included bees, such as *Bombus terrestris* and *Apis mellifera*, as well as the ground beetle *Poecilus cupreus*. At the trophic group level, comparisons of the “surrogate” species with other taxa showed that *T. pyri* provided a consistently protective representation for predators, whereas *A. mellifera* and *A. rhopalosiphii* did not always secure the full spectrum of sensitivity for pollinators and parasitoids, respectively. Notably, at least one

Tier 1 standard species (*T. pyri* or *A. rhopalosiphii*) consistently ranked among the most sensitive organisms for the tested compounds, supporting their continued relevance in testing schemes. Nonetheless, reliance on a single species may still underestimate effects within the broader trophic group, as observed for pollinators and parasitoids. Interestingly, ladybug larvae were consistently among the most sensitive organisms, in contrast to adults. Taken together, while current Tier 1 test species provide a practical foundation, expanding taxonomic coverage, accounting for life-stage differences, and refining surrogate species selection will be key to achieving more a realistic and protective pesticide risk assessment.

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2.11.T-03 Do Age and Exposure Route Make the Poison? Insights from a Multi-Route Pesticide Uptake Study with Ladybugs

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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., prey material for predaceous NTA), as well as direct exposure to the spray droplets during PPP-application (and spray drift), as discussed in the EFSA PPR Scientific Opinion on NTA risk assessment. The EFSA funded 2021-2025 project AENEAS set out to study the pesticide 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 ladybugs via (i) topical overspray, (ii) a dried residue on natural substrate surface (leaf or soil), (iii) dietary intake of oversprayed food items. Two binary mixtures of two active substances were tested on larvae and adult ladybugs (*Coccinella septempunctata*). After topical overspray, all four chemicals decreased from the exterior residues of larvae and adults to <20% within one day, and the volatile chemicals showed faster disappearance from the exterior residue. Internal residues were highest at the first sampling point of 2 h, and after that more slowly decreased from tissue. For ladybird larvae intake via contaminated food items was the dominant exposure route in the next five days studied, for ladybird adults the leaf residue contact and soil residue contact was the long term dominant exposure route. For both tested species residues for several exposure routes were above the 90-percentile Residue Unit Dose values measured in field studies. Topical overspray exposure results in a short term peak, and other exposure routes dominate the exposure profile depending on NTA species/lifestage/chemical. Based on these findings we suggest to take additional exposure routes into account in NTA risk assessment, while differentiating between organism groups.

2.11.T-04 Integrating GUTs Modeling into Ecotoxicological Assessments of Non-Target Arthropods in European Regulation

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The regulation of plant protection products in Europe mandates comprehensive environmental risk assessments for non-target arthropods (NTAs), which are essential for ecological safety evaluations. Over the past decade, effects modeling has gained recognition and has been incorporated into regulatory frameworks, for example in the recent European Food Safety Authority (EFSA) guidance on birds and mammals. However, current NTA test protocols are based on the International Organization for Biological Control (IOBC) guidance published in 2000, predating the development of effects models. Thus, these protocols are not specifically optimized to generate time-resolved data required for a robust parametrization of toxicokinetic-toxicodynamic (TKTD) models.

The General Unified Threshold model for Survival (GUTs) offers a framework for assessing survival under

chemical stress, incorporating temporal dynamics that traditional endpoints—typically measuring toxicant rates causing 50% mortality at a fixed time point—fail to address. We present a GUTs model specifically for *Coccinella septempunctata*, utilizing a data set that was originally produced for the registration of a plant protection product as a case study.

This presentation evaluates the compatibility of existing NTA testing methodologies with the GUTs framework, emphasizing implications for test design and data interpretation. We provide recommendations for enhancing the assessment and reporting processes in NTA ecotoxicological studies, advocating for adjustments that align with the GUTs approach. By bridging the gap between experimental ecotoxicology and effects modeling, this work aims to modernize the ecological relevance of risk assessments for NTAs in the context of plant protection product regulation.

Ultimately, this integration will contribute to more effective ecological risk management and regulatory decisions, ensuring the protection of non-target species as key components of agricultural biodiversity.

2.11.T-05 Risk Assessment for Off-Crop Habitats: Interactions Between Plant Protection Products and Non-Target Arthropods

Michael Peter Meissle, Marco Fioratti, Jörg Romeis and Vera Zina, Agroscope, Switzerland

Concerns about the impact of plant protection products (PPPs) on biodiversity persist, despite established tiered risk assessment frameworks for non-target arthropods (NTAs). Current approaches mainly address single active ingredient–NTA relationships within crops, leaving major knowledge gaps for NTAs inhabiting off-crop habitats. Semi-natural elements, such as hedgerows, flower strips, and field margins, are promoted to enhance biodiversity and ecosystem services, yet may also be exposed to PPP drift, potentially creating ecological traps. We present three complementary approaches to improve landscape-scale NTA risk assessment:

1. EU-wide biodiversity data compilation: We assembled a database of 130'000 NTA occurrence records linked to agricultural habitats either through systematic literature searches or by combining open-source biodiversity data with CORINE landcover information. This resource enables filtering by landcover type, environmental zone, taxonomy, and time to characterize species assemblages across agroecological contexts.

2. Systematic review of PPP–habitat interactions: A literature review on linear managed off-crop habitats examines biodiversity functions and PPP interactions (drift, residue dynamics, toxicity). Preliminary results highlight limited but increasing evidence on how these habitats mitigate or amplify PPP effects.

3. Field study on insecticide drift: A two-year experiment in Switzerland exposed flower strips to realistic acetamiprid drift doses. Short-term knockdown effects were observed within the first meter, especially for parasitic Hymenoptera, but no persistent population-level changes occurred across the 6-m-wide strips. Wider strips thus help buffer drift impacts, though minimizing drift remains essential.

These efforts provide critical building blocks for next-generation, holistic risk assessments that integrate spatial and temporal dynamics. Combining biodiversity databases, systematic reviews, and targeted field studies will reduce uncertainty and support more sustainable PPP use across diverse European landscapes.

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2.11.P Non-Target Arthropod Risk Assessment: A Simple Matter of Protection Goals or a Complex Web of Interactions?

2.11.P-We123 Risk assessment in an agronomic context – direct and indirect impacts of non-chemical management practices

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The risk to non-target terrestrial organisms (NTTO) from chemical plant protection products (PPPs) is well researched and the risks are evaluated during the registration process. However, chemical PPPs are only one set

of tools among a many other agronomic practices, supporting a range of agricultural activities that take place on-farm with the objective of optimising food production. These activities have the potential to either benefit or harm NTOs. Moreover, if PPPs are not available, the growers will take other actions to protect crops from pest and diseases. Consequently, changing one practice to manage one environmental risk or hazard can inadvertently impact other risks and even result in more harmful environmental outcomes. It is therefore important when considering the potential risk posed by PPPs to be able to put those into the context of the impacts presented by wider agricultural activities. Specifically, a solid knowledge of typical agronomic practices and the impact thereof on NTOs is essential 1) to take into consideration the agronomic reality when setting Specific Protection Goals for NTO for environmental risk assessments of PPP, and 2) to integrate a realistic agronomic baseline in future landscape models to address potential environmental risk by PPP.

In this poster we will present crop management calendars for two key European crops – wheat, representing annual arable production, and apples, representing perennial orchard production. We also examine differences in crop management between three different geographies from Northern Europe to the Mediterranean. The typical timing and frequency of key management practices and the proportion of the crop managed using them is evaluated, e.g. the proportion of the crop area subjected to inversion tillage vs. non-inversion tillage vs. direct drilling.

Via literature review, four regularly used non-chemical management practices (cultivation, mowing, harvesting and fertiliser use) are investigated in more depth to determine their impact on seven groups of non-target terrestrial organisms (earthworms, collembola, staphylinids, carabids, arachnids, lepidopteran larvae and soil mites).

The literature review covers direct impacts on abundance, diversity and biomass and longer term or indirect impacts such as recovery time and habitat changes.

2.11.P-We124 CropLife Europe Proposal for Specific Protection Goals for NTA Risk Assessment

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To approve Plant Protection Products (PPPs) under Regulation (EC) No 1107/2009, the absence of unacceptable environmental effects must be demonstrated. Specific Protection Goals (SPGs) translate this General Protection Goal into practical guidance for Non-Target Organisms risk assessment, including Non-Target Arthropods (NTAs). EFSA was mandated in 2024 to develop a NTA Guidance Document. To support this work, CropLife Europe experts have developed a proposal for SPGs for NTAs.

The first step in setting SPGs is identifying key Ecosystem Services relevant for agriculture provided by NTAs. Relevant regulating services provided by NTAs are pest regulation and pollination, which enhance agricultural production. Further supporting services such as habitat provision and nutrient cycling also play a role outside the production area. According to the EC mandate, SPGs are to be defined for the target application area managed by farmers (in-field) and areas outside the production area. In addition, the possibility of recovery as a normal ecological process, especially in disturbed habitats, which are typical for the agricultural landscape, should be considered when setting SPGs.

Further, when establishing SPGs it is essential to consider the agricultural context and to acknowledge that farming must be productive. The primary ecosystem service of the agricultural field is food production and consequently potential trade-offs with the protection of NTAs may arise and need consideration. In addition, the agricultural landscape is not a pristine environment and non-target organisms are constantly exposed to typical agronomic practices beyond PPPs (e.g. ploughing, harvesting, fertilization). These regular disturbances determine the spatio-temporal composition of NTA communities in agricultural landscapes. It is essential to take this agronomic context into account when setting SPGs to enable a translation of the SPGs into a fit for purpose, feasible, flexible and proportionate risk assessment framework.

SPGs for non-target terrestrial plants and soil organisms are also proposed and will be presented in separate posters at this conference.

2.11.P-We125 Developing an Impact Assessment Framework for Specific Protection Goals for Non-Target Arthropods

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In 2024, the EU Commission mandated EFSA to update the risk assessment methodology for the assessment of active substances and plant protection products (PPPs) with respect to their potential effects on non-target arthropods (NTAs) other than bees, soil organisms and non-target terrestrial plants (NTTPs). This process will require revision of the Specific Protection Goals (SPGs) and the associated Terrestrial Guidance Document that currently defines the risk assessment scheme for each organism class. The revision of the Terrestrial Guidance documents could lead to major changes in the agricultural practices and thus in important unintended socio-economic and environmental consequences. It is therefore relevant to evaluate the impact of these consequences and alternative options during the Guidance revision, as recommended according to the Better Regulation Guideline (2021).

In this poster, we present an impact assessment framework in which both environmental and socio-economic impacts are simultaneously addressed. The framework uses an ecosystem service (ES) approach to assess the impact of changes to Protection Goals and risk assessment scheme for NTAs (both in-field and off-field) on the availability of PPPs to growers, the alternative pest / disease control methods likely to be adopted by growers and wider socio-economic and environmental factors. This approach enables a holistic identification of trade-offs between changes in ES, the resulting adjustments to pest control methods used by growers and the broader consequences for food production, in terms of socio-economic and environmental costs and benefits (e.g. on sustainable productivity for EU farmers including the ability to handle pest outbreaks). In addition, a more targeted investigation, gaining insight from EU agronomists resulting in a series of crop and country-specific case studies, collates the predicted effects and changes to agronomic management practices that would result from changes to SPGs. The proposed framework allows evaluation of the impact of different SPGs and risk assessment options on ES provided by NTAs and agricultural land in terms of socio-economic and environmental costs and benefits. The outcome can contribute to the necessary trade-off decisions while evaluating alternative SPG options.

2.11.P-We126 Non-Target Arthropods (NTA): Specific Protection Goals, Indirect Effects and Ecosystem Services

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Higher-tier NTA studies assess how local communities respond to test item exposure within highly complex natural systems. Evaluating species abundances in isolation is considered insufficient for predicting the strength of an ecosystem as emergent or counterintuitive effects often arise from interactions within community networks. Within an ecosystem, organisms fulfil specific functions—such as pest control or pollination—through their interactions with other organisms and with the environment; these functions reflect their ecological roles. Such ecosystem services relate directly to the specific protection goals defined by EFSA.

Here, we do not focus on the direct effects of PPP applications on single species. Instead, we shift attention to species interactions and to the indirect effects of PPP applications through interactions within an NTA community.

In the first step, we analyse community composition and the proportions of different ecological roles in NTA communities across several habitat types (off-crop, in-crop, apple orchard). We then examine if and how these proportions change over time, both with and without PPP application.

In the second step, we concentrate on selected pest species and their principal predators. We investigate their population dynamics over time, with particular emphasis on indirect effects of PPP applications on predatory species that are not the intended targets of an intervention.

Overall, shifting the perspective from single-species assessments to a community-level approach—including explicit consideration of species interactions—is essential for achieving sustainable pest management and maintaining key ecosystem processes.

2.11.P-We127 Classification and Risk Assessment of Terrestrial Arthropods in European Agriculture: Integrating Ecological Diversity within the Revised NTTO Guidelines

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Regulatory frameworks for plant protection products (PPPs) mandate thorough assessments of their safety to non-target arthropods. These assessments ensure that agrochemicals do not adversely affect beneficial insects, crucial for ecosystem functions like pollination and pest control. We present a trait-informed framework for classifying terrestrial arthropods into ecologically meaningful groups to support ecological risk assessment (ERA) and inform trait-based modeling approaches, thereby enabling future population-level risk assessments.

Classification of terrestrial arthropods in European agricultural landscapes is based on taxonomy, habitat, movement behavior, diet, life cycle, and overwintering strategies complemented by representative species. Using the Pop-GUIDE approach, we developed matrices for major groups (e.g., ground-active predators, pollinators, foliage herbivores) detailing model objectives (persistence, connectivity, acute risk), trait precision levels (General, Realistic, Precise), exposure pathways, spatial/temporal resolution needs, and uncertainty drivers. Each profile also includes key life-history traits and ecological interactions relevant for ERA endpoints such as dose-response, TKTD, and indirect effects. Among these ecological groups, ground-active predators and omnivores (Carabidae, Araneae) play significant roles in prey dynamics, while foliage-dwelling herbivores (Lepidoptera, Hemiptera) highlight the risks posed by agricultural inputs. Pollinators (Apidae, Syrphidae) illustrate the connectivity vital for ecological functioning, and parasitoids (Hymenoptera) are essential biocontrol agents.

This structured approach enables prioritization of surrogate species and supports the development of group-specific population models under the revised EFSA NTTTO guidance mandated by the European Commission to the European Food Safety Authority (EFSA) in 2024. By linking ecological traits to modeling requirements, it improves realism in risk assessments and promotes sustainable management strategies. The framework addresses regulatory demands for representative surrogate species across NTA taxa, ensuring sufficient conservatism and integration of ecological data into agricultural risk management.

By providing a comprehensive classification of non-target arthropods, this research supports informed decision-making for biodiversity conservation and the protection of ecosystem services in agricultural landscapes.

2.11.P-We128 NTA Field Studies: Effect of Sampling Effort on Non-Target Arthropods (NTA) Field Data

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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. To ensure a robust risk assessment, it is essential to sample a representative portion of the NTA community with respect to both diversity and abundance. Arthropod abundance is particularly important because it determines the strength and reliability of statistical analyses. However, sampling effort is constrained by practical considerations: although increasing the number of replicates generally improves data quality, there is a threshold in terms of feasibility due to limitations in e.g. field size, labour, and cost.

Here, we evaluate how sampling effort influences (1) NTA community composition (diversity and abundance) and (2) the number of taxa available for population-level statistical analyses. Specifically, we examine how sampling between 3 and 12 replicates in apple orchards and between 3 and 28 replicates in meadow systems affects the taxonomic richness (diversity and abundance) captured. As replication increases, the number of taxa reaching sufficient abundance for statistical evaluation is expected to rise until reaching a saturation point. Our goal is to determine where this saturation occurs and whether such replication levels are practically achievable in routine field studies.

Additionally, we (3) compare the taxa that achieve adequate abundance for analysis with the list of focal taxa recommended for inclusion in NTA studies by De Jong et al. (2010). Special attention is given to how replicate number influences variability—and therefore the robustness—of the dataset. Using this approach, we evaluate how good the commonly used four-replicate design is for detecting less abundant taxa, how many additional taxa can become available for population analysis with increased replication, and whether such increased sampling intensity provides an advantage for the risk assessment while keeping a cost-effective approach.

Reference: De Jong, F.M.W. et al 2010. Guidance for summarising and evaluating field studies with non-target arthropods. RIVM report 601712006/2010. 76 pp.

2.11.P-We129 Developing a Framework For Identifying Sentinel Taxa for the Non-Target Arthropod Risk Assessment in Europe Using Field Effect Studies – A Case Study with Bembidion

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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, abundances

and phenologies, 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. We present a case study using *Bembidion* ground beetles, highlighting both the challenges of identifying sentinel taxa but also possible solutions including pooling of species based on analysis of traits and chemical sensitivity.

Disclaimer/Disclosure:

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2.11.P-We130 From Field Variability to Regulatory Reliability: Rethinking Spider Testing in PPP Assessment

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Spiders of the genus *Pardosa* (wolf spiders) are ecologically significant non-target arthropods widely present in European agroecosystems. As generalist predators, they contribute to pest regulation and biodiversity, making them valuable indicators in environmental risk assessments for plant protection products (PPPs). To address this, the International Organisation for Biological and Integrated Control (IOBC/WPRS) developed an acute contact toxicity test for *Pardosa* spp., which is commonly used in tiered terrestrial ecotoxicity assessments under EU Regulation (EC) No 1107/2009. However, concerns have emerged regarding the reproducibility and regulatory robustness of this guideline compared to Organisation for Economic Co-operation and Development (OECD) terrestrial protocols, such as Test No. 207 for earthworms.

This study critically evaluates the *Pardosa* guideline, highlighting methodological inconsistencies and proposing harmonization with OECD standards. A comparative analysis shows that while spider and earthworm tests share core elements—such as mortality and body weight endpoints—the spider test suffers from overly stringent and arbitrary validity criteria, particularly for control mortality thresholds. These criteria often invalidate tests due to natural variability in field-collected populations, exacerbated by sex-related differences and seasonal effects. A case study of three control tests demonstrated male mortality rates far exceeding guideline limits (up to 59%), while females consistently showed 0% mortality, indicating that mixed-sex controls introduce unnecessary variability.

Statistical modeling using binomial probability supports replacing fixed percentage thresholds with confidence-based criteria, advocating a 10% mortality limit as statistically defensible. Additional refinements include sex-specific testing, standardized acclimation and feeding protocols, and the use of artificial soil to improve ecological relevance. Aligning the spider test with OECD principles would enhance methodological consistency, reduce invalidations, and ensure data comparability across studies.

This work underscores the need for guideline revision to strengthen reliability and regulatory acceptance. Implementing these changes will facilitate robust datasets for ecological modeling and PPP risk assessment, ensuring that testing protocols reflect both ecological importance and regulatory rigor.

2.11.P-We131 In-field exposure of non-target arthropods in the litter layer of treated crops

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In agricultural fields, pesticides are typically applied spraying downward onto standing crops, where they adhere to plant surfaces and the soil before reaching NTAs in the litter layer. Ground-dwelling NTAs may be exposed directly through overspray or subsequently through contact with wet formulations, dried residues on vegetation or soil, or by ingesting contaminated plant material, prey, or other surface substrates. Since plant structures intercept much of the spray, deposition typically decreases from the crop canopy toward the soil, leaving soil-dwelling NTAs less exposed than organisms higher in the canopy. However, it remains unknown what proportion of the pesticide that reaches the soil ultimately adheres to soil-dwelling NTAs. To address this gap, we conducted an experiment in a rapeseed field to measure how pesticide deposition declines along the plant's vertical profile before reaching the soil and sampled soil-dwelling NTAs using pitfall traps. The field was sprayed with a non-toxic fluorescent dye (Acid Yellow), using standard agricultural application techniques. The vertical distribution of the chemical in the crop was measured by placing four glass-fiber filter discs at different plant heights (100, 60, 30, and 0 cm above the soil). Pitfall-traps were emptied on two consecutive sampling days at 4- and 16-hours post-spray. Exposure decreases by more than fivefold from the top of the canopy down to the soil layer, with the decline being gradual near the upper canopy and much steeper in the lower canopy. NTAs captured 4 hours after spraying were on average 2.5 times less exposed than measurements of soil-level filters would suggest, while individuals captured 16 hours after spraying were 4 times less exposed than soil-level values from the initial application on the previous day. These results show that actual exposure of ground-dwelling NTAs is much lower than expected from the initial amount of chemical sprayed per hectare (kg/ha) and even lower than actual measured levels on ground level (taking crop inference into account).

Disclaimer/Disclosure:

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2.11.P-We132 Clarifying Terminology for Off-Field Risk Assessment: Definitions of Edge-of-Field and Field Margin

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Under Regulation (EC) No 1107/2009, the approval of Plant Protection Products (PPPs) requires demonstrating the absence of unacceptable environmental effects. This overarching General Protection Goal is operationalized through Specific Protection Goals (SPGs), which provide practical guidance for risk assessment of Non-Target Organisms (NTOs). In 2024, EFSA was mandated to revise and update its existing Guidance Document to ensure SPGs remain scientifically robust and aligned with current regulatory needs.

SPGs are inherently linked to spatial scale and location. Nevertheless, clarity regarding the extent and positioning of the spatial scale and location of an SPG is lacking, which is mostly due to the fact that used terms are not clearly defined. This ambiguity complicates exposure modeling and mitigation strategies. One critical aspect is the delineation of edge-of-field and field margin, which plays a pivotal role in environmental risk assessment.

Clarifying these spatial concepts is essential because it underpins SPG discussions and supports the development of spatiotemporally explicit models (e.g., xOffFieldSoil) and tiered risk assessments that integrate realistic exposure patterns and mitigation measures.

To address this, experts from CropLife Europe (CLE) have developed a proposal to harmonize definitions. The adoption of standardized definitions for terms such as “field”, “edge-of-field” and “field margin” will:

- Enhance regulatory consistency across Member States.
- Improve comparability of risk assessments.
- Strengthen the scientific basis for protecting off-field habitats and biodiversity within agricultural landscapes.

2.11.P-We133 Simulated Drift in Flower Strips – Effects on Non-target Arthropods

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Plant protection products (PPP) can reach off-crop habitats through spray drift and potentially affect non-target arthropods (NTAs). Flower strips are increasingly established to support biodiversity and ecosystem services in agricultural landscapes, yet their placement along cropped fields exposes them to PPP drift. Despite their relevance, empirical data linking realistic drift exposure to population- and community-level responses in these habitats remain limited. This study addresses two related questions: how much insecticide reaches flower strips and how do these drift levels affect resident NTA communities?

In 2022, tracer-based drift measurements quantified insecticide deposition on 6-m flower strips in Tänikon, Switzerland. While deposition reached up to 28% of the field rate in the first meter, it declined sharply with distance. These field-derived values were then used to design a two-year drift simulation experiment at the same location (2023-2024). Annual flower strip plots in a randomized block design were exposed to simulated Drift-rates of the broad-spectrum insecticide acetamiprid (High: 28%, Medium: 1.2%, Low: 0.4%), alongside with untreated controls and Full-rate positive controls. The NTA community was monitored before and after applications from June to August. Short-term effects were assessed using dry pan traps (knockdown) and sticky traps (escape and movement). Suction sampling and pitfall traps were used to quantify longer-term population responses and recovery.

Drift-rate exposure caused short-term knockdown effects mainly within the first meter of the flower strip, affecting several taxa including parasitic Hymenoptera. No persistent changes at the population or community-level were detected across the 6-m flower strips over the season. In contrast, Full-rate treatments caused strong and sustained reductions in multiple taxa, confirming the sensitivity of the monitoring design. NTAs exposed to simulated Drift-rates of acetamiprid recovered rapidly during the season, with no indication of cascading or sustained impacts.

By integrating measured deposition patterns with controlled drift simulations, this study provides a field-validated basis for evaluating realistic off-crop exposure scenarios. The findings suggest that wide flower strips (? 6-m) can buffer local drift impacts, while highlighting the first meter as a critical zone where drift reduction measures remain essential.

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2.11.P-We134 Reviewing Toxicokinetic Determinants of Pesticide Sensitivity in Invertebrates: a Bridge between Target and Non-Target Species

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Species sensitivity to chemicals is determined by a complex combination of factors that influence the chemical exposure and its biological effects. Pesticide properties and species-specific traits may affect toxicokinetic processes, as well as toxicodynamics. Sub-organismal features in invertebrates can influence pesticide toxicokinetics and may contribute to interspecies sensitivity differences. Adaptation to repeated pesticide exposure can lead to resistance as the organism (usually the target pest) produces more or effective systems to tolerate the pesticide. While the molecular determinants of resistance and sensitivity in target arthropods are well studied, those in non-target invertebrates remain poorly understood, often due to experimental challenges and limited availability of suitable methods. Mechanistic approaches in ecotoxicology are limited to a few model species due to their relevance in environmental risk assessment, but their extrapolation power to other invertebrate species is still low. In this review, we summarize current research on sub-organismal, toxicokinetic-related mechanisms in target and non target species and identify molecular endpoints that can be translated between resistance and sensitivity research.

We conducted a Web of Science search to identify (eco)toxicology studies on invertebrates, pesticides, and toxicokinetic-related molecular endpoints. The literature was categorized based on the investigated process (Uptake, Distribution, Detoxification, Elimination). The uptake and distribution categories yielded few results in terms of sub-organismal methods. In elimination and detoxification research, a broad range of molecular methods was applied to study resistant pest, with methods often performed in combination. Network analysis showed that sensitivity studies most often pair gene expression with enzyme activity measurements, while resistance studies combine multiple molecular approaches.

The methodological divide found in our search reflects a broader disconnect between research communities studying target and non-target organisms. Contributing factors include different vocabulary, geographical biases, and weak academia-industry partnerships.

Despite shared processes underlying pesticide toxicity, molecular tools routinely applied in pest research are

rarely applied to non-target invertebrates. Filling these gaps requires access to annotated genomes, standardized methods, and taxonomically broad data generation.

2.11.P-We135 Triazole Fungicides: New Disruptors of Hormonal Signaling in Coleopterans

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The application of pesticides, especially triazole fungicides, contributes significantly to biodiversity loss in agroecosystems. Although their mode of action targets the inhibition of ergosterol synthesis in fungi, in arthropods they may interfere with the production of ecdysteroids, hormones essential for molting and reproduction. Given the key role of invertebrates in maintaining plant biodiversity and in trophic networks, it is crucial to understand how these compounds affect their physiology. In this study, the effects of prothioconazole and tebuconazole were evaluated in the beetle *Tenebrio molitor*, reproducing a field-like exposure through the ingestion of treated cereal seeds.

Three treatments were used: untreated seeds, treated seeds, and a 50:50 mixture. For ten days, larvae consumed only these food sources, and their weight, consumption, development, mortality and potential malformations were recorded. In addition, the expression of 13 genes related to the ecdysteroid pathway was analyzed using RT-qPCR, and levels of 20-hydroxyecdysone and cholesterol were quantified. Models of the ligand-binding domain of the ecdysone receptor (EcR) were also generated to perform molecular docking studies with triazoles and known EcR-binding molecules.

The results showed a drastic reduction in food consumption in larvae exposed to Raxil® Plus and a significant decrease in larval weight, although no subsequent effects were detected in pupae or adults. No alterations in development, mortality or morphology were observed. However, exposure caused a strong decrease in the expression of key ecdysteroidogenesis genes in larvae, as well as a marked downregulation of HR3 and overexpression of E75. Larval ecdysone levels were also significantly reduced, while cholesterol levels showed no variation. Molecular docking analyses indicated that triazoles displayed binding similar to compounds with known affinity for EcR in the non-steroidal conformation.

Overall, the data demonstrate that triazoles, even at environmentally realistic levels, alter growth, hormonal regulation and ecdysone receptor function in *T. molitor*, suggesting potential impacts on beetle populations and other arthropods in agricultural environments.

2.11.P-We136 A First Modelling Approach to Predict Behavioural Alterations in *Porcellionides pruinosus*

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Behavioural alterations in terrestrial isopods emerged as early warning signals of soil pollution, but their link to specific physicochemical properties of contaminants remains unexplored. Clarifying which chemical properties predict behavioural disruption is essential for advancing ecotoxicological assessment. This study aims to examine the correlation between a selection of physicochemical properties of chemicals and the onset of behavioural changes in *Porcellionides pruinosus* population. Behavioural effects were assessed performing the avoidance bioassay, which reflects active migration away from contaminated soil, in combination with the disaggregation effects, which reflects the alteration of social behaviour of woodlice. Twenty-five synthetic organic chemicals, with different modes of action, and representing three use categories (plant protection products, pharmaceuticals and personal care products, and perfluorocarboxylic acid) were tested. Several nominal concentrations expressed in mg/kg dry soil (d.w.) were selected based on the respective maximum solubilities (g/L) of each compound, in order to avoid the use of organic solvents that could have masked or affected the behavioural responses and the related study of physicochemical descriptors. The behavioral bioassays were carried out in thermostatic chambers at 20 ± 1 °C, with a 16:8 h (light:dark) photoperiod, for 48 h. Based on the outcomes, chemicals were classified into three categories: those inducing both avoidance and disaggregation, those eliciting only one response, and inactive substances within the selected concentrations. Regression models revealed a significant positive association between disaggregation and Henry's law constant, with a threshold of 1×10^{23} Pa·m³·mol⁻¹, with 95% confidence interval estimated using the Delta method, above which aggregation breaks down. This finding suggests that Henry's law constant may serve as a potential, though yet to be validated, predictor in regulatory contexts for assessing the impact of chemicals on gregarious populations. No predictive descriptors were identified for avoidance, indicating that further research is required to elucidate the factors driving migration. Furthermore, in view of the heterogeneity of behavioural responses,

the combined use of avoidance and disaggregation endpoints is recommended for a more comprehensive investigation.

2.11.P-We137 Developing a Generic Exposure Model for Leaf-Feeding Lepidopteran Larvae To Support Non-Target Arthropod (NTA) Risk Assessment (RA)

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Non-target Lepidopteran larvae are increasingly recognized as vulnerable recipients of plant protection product (PPP) exposure on treated foliage. The EFSA scientific opinion on NTA RA recommends including oral toxicity studies for Lepidopteran larvae as representatives of herbivorous NTAs in PPP RA for the upcoming revision of the terrestrial Guidance document. In order to use existing and future oral toxicity data for Lepidopteran larvae in risk assessment, a transparent exposure model to derive daily food intake and corresponding PPP dose is required. We present the conceptual design of such a model, which is currently under development for leaf-feeding Lepidopteran larvae. The model builds on work by the German Environment Agency (UBA) on the threatened butterfly *Parnassius apollo vinningensis* (Stichel 1899), the Mosel Apollo, an endemic subspecies of the Apollo butterfly restricted to steep vineyard slopes in the Mosel river region. In this case study, the key question was whether PPP deposition on larval host plants may pose an unacceptable risk, thereby potentially contributing to the imminent extinction of this subspecies. Motivated by this example, we develop a more generic exposure model for leaf-feeding Lepidopteran larvae. Larval development is represented on a relative time axis and combined with literature-based growth curves and simple allometric relationships between body mass and food intake, with explicitly increased feeding in late instars. By coupling predicted daily food intake with residue levels on host plants, the model provides estimates of daily PPP dose to caterpillars for different larval groups (for example small/medium/large species, four to six larval instars). We will present the model framework, key parameter choices and parameterization using published data for several Lepidopteran species taking into account uncertainties. Planned applications include (i) screening-level risk characterisation for caterpillars based on existing oral toxicity data for Lepidoptera or other insects, and (ii) sensitivity analyses exploring how assumptions on growth and feeding behaviour affect exposure estimates. The model is intended as a pragmatic, transparent tool to help address current gaps in non-target arthropod risk assessment for Lepidopteran larvae and to support future refinement of guidance for non-target Lepidoptera.

2.11.P-We138 Application of the BufferGUTS model to determine toxicity thresholds for a set of 16 NTA species as key drivers for ecosystem services

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For a set of 16 NTAs species, representing the trophic groups of predators, pollinators and parasitoids, laboratory toxicity tests using three pesticide commercial formulations with different modes of action were performed: Karate Garden (a.i., lambda-cyhalothin), Sanium (a.i., flupyradifurone), and Mainspring (a.i., cyantraniliprole). Test organisms were exposed to the products by a single direct overspray application using a Potter spray tower. After exposure, the oversprayed organisms were transferred to incubation cages, supplied with food ad libitum, and effects were assessed 4, 20, 24, 28, 44 and 48h after exposure and then every 24h until the end of the test. For the interpretation of acute test dose-response data, we used the recently published BufferGUTS model. Nominal application rates application rates (ng a.i. cm⁻²) and raw observations of survival over time were used to calibrate the BufferGUTS model using the implementation in the MemPyGUTS Python package hosted by Osnabrück University. The results of the model calibration to raw observations of survival over time resulted in a set of BufferGUTS parameter values including uncertainties. Using the calibrated model, lethal rate (LR50) values after 48 and 96 hours were calculated.

Comparison to experimentally derived LR50 values shows that lethal doses after 48 hours tend to be very similar using the BufferGUTS model but show smaller uncertainties. The matching of model-derived with data-derived LR50 values confirms the realism of the BufferGUTS model, while the smaller uncertainties of the

model-derived values indicate that the model is using more data points. The extrapolation of LR50 values to 96 hours observation time indicates that the toxicity thresholds are expected to generally decrease for a prolonged observation time. A more detailed analysis reveals that a simulated prolonged exposure time has different consequences for the LR50 values for three tested PPPs and the tested species. For Mainspring, for 11 species the LR50 values were reduced by about 50% of the values after 48 hours, while for Sanium and Karate this was the case for 5 species.

These calculations show the potential of using GUTS modelling for the analysis of results from toxicity testing. Once calibrated, these models can be used to predict toxicity thresholds for inter- or extrapolated exposure times.

2.11.P-We139 Identification of Biomarkers for Immunosuppression in PAMP-challenged *Tribolium castaneum* larvae using Transcriptome Analyses

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It is assumed that in stressed ecosystems, pathogens exert strong pressure on populations. In addition, the organisms in the environment are exposed to a large number of environmental pollutants such as pesticides or drugs. The ecotoxicological hazard and risk assessment of chemicals currently do 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 project aims at the identification of reliable markers for the MoA of immunomodulatory chemicals in Red Flour Beetle larvae (*Tribolium castaneum*). For this, exposure by a chemical with a known mode of action (MoA; immunosuppressive or non-immunomodulative) will be followed by an immune challenge. This setting enables the investigation of chemical effects in the activated immune system of the beetle larvae. The exposure chemical will be mixed with the food of the larvae, a flour-yeast-mixture, and the larvae are exposed for five days ad libitum. After the exposure, the larvae are challenged via injection of a Pathogen-associated-molecular-pattern (PAMP) that induces an immune system activation. 6 h later the RNA of the larvae is extracted and the transcriptome is analyzed. The analysis of the total RNA enables a clear differentiation of chemical MoA in the resting as well as the activated immune system. With this approach, we will be able to gain insight into the immune system of the invertebrate species *Tribolium castaneum* and to define marker genes for the discrimination between immune-modulating chemicals and other MoA.

2.11.P-We140 Determination of Immuno-Ecotoxicological Effects on the Ground Beetle Species *Poecilus cupreus* under Lab- and Landscape Conditions

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Climate change, habitat fragmentation, and invasive species represent major challenges for ecosystems and their stability, exerting significant pressure on the organisms living within them. This increased pressure makes it easier for additional factors, such as pathogens or the growing contamination by various chemicals and fertilizers, to cause harmful effects and add further stress to these organisms. Currently, multiple stressors and their effects on the immune system are either not considered or only insufficiently addressed in the approval process of substances. However, it is well known that chemicals, such as pesticides, can have numerous effects on immune-specific factors and, in combination with pathogen pressure, may destabilize ecosystems. Therefore, it is essential for the approval and restriction of substances used by humans and released into the environment to identify immuno-ecotoxicological modes of action (MoA) and determine their current impact on ecosystems. The aim of this project is to compare the immune status of free-living ground beetles with that of laboratory-reared individuals of the same species, and the model organism *Tribolium castaneum* (red flour beetle). The study focuses on the ground beetle species *Poecilus cupreus* (copper greenclock beetle), which is of high ecological relevance due to its presence in a wide range of habitats, its extensive research history, and its suitability for laboratory maintenance and breeding. Laboratory beetles and larvae are treated with

immunosuppressive and immunostimulatory chemicals with known MoA and subsequently injected with pathogen-associated molecular patterns (PAMPs) to activate the immune system from its resting state. This process, also called "challenge", and the immune system's response to the treatments are detected and identified through transcriptomic and proteomic analyses. Special attention is given to parameters and molecular reactions that affect the immune system and lead to changes within the organism. These reactions in laboratory-bred beetles will then be compared to the molecular immune responses of free-living beetles, ultimately enabling an assessment of the immuno-ecotoxicological status of individual habitats and facilitating the transferability of findings to other locations.

2.12.A Terrestrial Biodiversity and Chemical Pollution: Understanding Exposure and Impacts Towards Improving Risk Prevention and Management

2.12.A.T-01 Occurrence and Distribution of Organic Micropollutants in the Barn Owl Food Chain: A Pan-European case study

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Organic micropollutants pose significant threats to terrestrial ecosystems by disrupting biodiversity, ecosystem structure, and function. Understanding the extent and pathways of chemical pollution in these systems is critical for effective environmental management. The EU-funded TerraChem project addresses this challenge through an integrated systems approach, combining advanced monitoring, modeling, and analytical technologies to map chemical exposure across multiple trophic levels in European terrestrial habitats. This study contributes to TerraChem by investigating organic micropollutant occurrence and biomagnification within the barn owl (*Tyto alba*) trophic chain across six European countries (Netherlands, Germany, Spain, Portugal, Greece, Romania). Samples of barn owl livers, rodents, invertebrates, plants, and soils were collected and analyzed using optimized multi-matrix extraction protocols including Accelerated Solvent Extraction and ultrasonic-assisted extraction, followed by high-resolution and low-resolution mass spectrometry techniques (LC-TIMS-HRMS, GC-HRMS, GC-MS/MS). Results reveal widespread detection of per- and polyfluoroalkyl substances (PFAS), with perfluorooctanesulfonic acid (PFOS) ubiquitously present in all barn owl livers, demonstrating bioaccumulation and biomagnification along the food chain. Additionally, second-generation anticoagulant rodenticides such as brodifacoum showed high concentrations particularly in owls and rodents, reflecting trophic transfer from prey to predator. A suite of plant protection products (including insecticides, fungicides, and herbicides) were also identified, with the lowest contamination in plants, indicating differential exposure across trophic levels. This evidence underscores the barn owl's value as a biomonitor for assessing environmental organic contaminants and highlights complex exposure pathways. The findings emphasize the need for sustained surveillance and comprehensive chemical risk management to protect terrestrial biodiversity and ecosystem services, supporting the EU's zero pollution ambitions through data-informed regulatory frameworks.

2.12.A.T-02 A new systems approach addressing chemical risks to terrestrial biodiversity in Europe

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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:

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2.12.A.T-03 A Historical Perspective on Bioaccumulation Research and Regulations

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At present, most government jurisdictions around the world use bioaccumulation screening criteria that represent chemical exposure to aquatic water-breathing organisms, which includes the bioconcentration factor (BCF) and bioaccumulation factor (BAF) in fish and the octanol-water partition coefficient (KOW). These metrics are limited in their ability to assess bioaccumulation in organisms because they only assess bioaccumulation in aquatic water-breathing organisms and are not representative of bioaccumulation in many wildlife species or classes of species such as birds and mammals that are air-breathing organisms (Kelly et al., 2007). The continued use of BCFs to assess bioaccumulation potential is astounding from an historical perspective since birds and marine mammals were found to be the most impacted by the dirty dozen of organochlorines that gave rise to environmental regulations of substances and from a current modern perspective where substances of current interest like many perfluoroalkyl substances (PFAS) have low BCFs in fish, low or no KOW, but are highly bioaccumulative in birds and mammals. BCFs and BAFs also do not represent or assess biomagnification in organisms. Hence, field-based biomagnification and trophic magnification factors (BMFs and TMFs) are regarded by many experts as more appropriate metrics for assessing biomagnification potential than the BCF and BAF (Gobas et al., 2009; Borgå et al., 2012; Burkhard et al., 2012). This expert advice has now been incorporated into the recent Guidelines on Information Requirements and Chemical Safety Assessment (European Chemicals Agency, 2023), which has made BMFs and TMFs more prominent tools for bioaccumulation assessments. This opens the door to the use of wildlife data for regulatory purposes. However, the use of wildlife data has been viewed with considerable wariness because of the inherent variability and uncertainty of real-world bioaccumulation data and the challenging conditions for replication. The purpose of this presentation is to discuss the historical context of the bioaccumulation regulations and to highlight a path forward for the use of wildlife data for regulatory bioaccumulation assessment.

2.12.A.T-04 Linking Pesticide Mixture Toxic Pressure to Species Loss in European Agricultural Soils

Susan Oginah, **Farshad Soheilifard** and **Olivier Jolliet**, *Quantitative Sustainability Assessment, Technical Univ. of Denmark, Denmark*

Predicting the damage caused by chemical mixtures to structural biodiversity is relevant for conducting integrated chemical risk assessments and protecting biodiversity. A robust relationship has been established between the effects of toxic pressure mixtures and species loss in aquatic ecosystems. However, we still lack such refinement in terrestrial ecosystems due to data gaps. To address this gap, we began by combining modelled pesticide exposure data from the Pangea model with approximately three million species occurrence records from the Global Biodiversity Information Facility (GBIF), which were harmonized at a 10×10 km grid. A partial mixture toxic pressure was derived from 133 of 900 substances, expressed as summed hazard units (like PAF, the potentially affected fraction of species), based on chronic EC10 endpoints and aggregated across substances per site. To explore the relationship between toxic pressure and biodiversity loss, we applied Generalized Additive Models (GAMs) to predict the potentially disappeared fraction (PDF) of species as a function of increasing toxic pressure. Our preliminary results indicate that species richness declines with increasing toxic pressure, with no observations of high richness at high exposure levels. The GAM model shows that a 20% change in mixture toxic pressure results in 25% of the present species disappearing. Thus, we determined an extrapolation factor of a near 1:1 relationship between predicted effects and actual damage required for different decision-support tools, such as Life Cycle Assessment. The current results represent only partial estimates and are based on agricultural soils with frequent chemical inputs; they may overestimate the biodiversity impact compared to that in natural or semi-natural soils. We will incorporate biomonitoring data from WP1 in the TerraChem project and expand the scope to include other substances beyond pesticides. This will enable the development of a robust PAF-to-PDF relationship for the terrestrial ecosystems, supporting the implementation of improved chemical risk prevention and management strategies.

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2.12.B Terrestrial Biodiversity and Chemical Pollution: Understanding Exposure and Impacts Towards Improving Risk Prevention and Management

2.12.B.T-01 Relating Chemical Effects to Ecosystem Functioning: Introducing the Functional Sensitivity Distribution

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1. Introduction

Current approaches for estimating the effects of chemical exposure commonly rely on species sensitivity distributions, which focus on species richness. However, the concept of biodiversity is much broader, and the species sensitivity distribution does not consider the functional role species have within ecosystems. Functional richness, based on species traits, provides a more direct relation to ecosystem functioning. In this study, we developed the “functional sensitivity distribution” to examine chemical effects on functional richness and compared it with the species sensitivity distribution.

2. Materials and Methods

To assess the effect of chemicals related to ecosystem functioning, we adapted the species sensitivity distribution by replacing species richness with functional richness. Both trait data and ecotoxicity data served as inputs. Functional richness was calculated by mapping traits in a multidimensional space and determining the volume within. Firstly, we calculated the base functional richness at the absence of a toxicant, thus with all species. Next, we used the ecotoxicity data to determine at which concentration a species was affected. Once affected, we excluded this species from the calculation and recalculated functional richness without that species. This process was continued until all species were affected. We then determined the potentially affected fraction of functional richness. Lastly, we constructed the functional sensitivity distribution by fitting a logistic curve through all data points.

3. Results and Discussion

Both species richness and functional richness declined with increasing chemical concentration. While the species sensitivity distribution showed a uniform decline, the functional sensitivity distribution revealed a stepwise decline. Functional groups showed redundancy, and notable changes occurred only when the last species within a group was affected. Therefore, both methods together enhance understanding by providing information on the effects on biodiversity and its implications for ecosystem functioning.

4. Conclusions

The functional sensitivity distribution revealed patterns like the species sensitivity distribution but added insight into how effects on species relate to functional change. Together, these methods provide a more robust basis for chemical risk assessment and can support policymakers in setting safe background concentrations.

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2.12.B.T-02 Are pesticides a relevant driver of terrestrial biodiversity loss?

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Agricultural intensification is a major driver of biodiversity decline in Europe, and pesticides are increasingly discussed as an important contributing factor. However, it remains unclear whether current environmental risk assessment (ERA) sufficiently captures their effects on non-target organisms at population and higher levels. This study synthesises quantitative evidence on the effects of currently used pesticides in Europe on terrestrial biodiversity.

We conducted a literature search for the period 1.1.2015–1.5.2025 and restricted inclusion to syntheses, systematic reviews and meta-analyses addressing synthetic organic pesticides currently authorised in at least one EU Member State, with effects on non-target organisms relevant for Europe and reported at population level or above. Narrative reviews, studies focusing only on sublethal endpoints, and clearly non-authorised legacy substances were excluded. Titles and abstracts (10,798 unique records) were screened using a machine-learning assisted tool (ASReview 2.1.1), applying stopping rules (?7.5% screened and ?100 consecutively predicted non-relevant records). Deep screening further required environmentally realistic exposure, clear attribution to agricultural pesticide use in field studies, systematic literature coverage, and at least five observations from ?3 primary studies.

This process yielded 10 publications comprising 51 effect-size cases. Of these, 39 effect sizes were negative, seven indicated no effect and five were positive. Restricting to statistically significant cases (95% confidence intervals not overlapping zero) resulted in 17 effect sizes, all negative. For studies that exclusively considered current-use pesticides, the seven effect sizes were negative. Mean log response ratios were generally negative. Insecticides showed the strongest negative effects on animals and soil invertebrates, whereas herbicides most strongly affected microorganisms and plants. The results demonstrate consistent negative effects of pesticides, including currently authorised substances, on terrestrial biodiversity. These findings challenge the assumption that current ERA approaches are broadly protective at ecosystem level and highlight the need to better align regulatory thresholds with empirically observed biodiversity impacts.

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2.12.B.T-03 Protecting Terrestrial Biodiversity Through Chemical Regulation: A Critical Review of European Environmental Risk Assessment Frameworks

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Chemical pollution is one of the five main drivers of biodiversity loss, yet European chemical regulatory frameworks provide fragmented and inadequate protection. This systematic review examines how REACH, CLP, Biocidal Products Regulation, Plant Protection Products Regulation (PPPR), and pharmaceutical regulations integrate biodiversity into Environmental Risk Assessment (ERA) through comprehensive analysis of legal texts and technical guidance documents.

Findings reveal critical gaps: only PPPR and Biocidal Products Regulation explicitly designate biodiversity as a protection goal. Terrestrial species assessment is severely limited—birds and mammals receive only in some frameworks routine evaluation while amphibians and reptiles remain largely unassessed. Risk assessment relies predominantly on single-species toxicity endpoints, with population-, community-, and ecosystem-level effects absent. No regulation systematically applies Essential Biodiversity Variables across genetic, species, and ecosystem levels. Additional deficiencies include inadequate assessment of transformation products, insufficient mixture toxicity evaluation and integration, and systematic failure to integrate environmental monitoring data into risk management.

We conclude that current ERA schemes inadequately protect terrestrial biodiversity due to: (1) missing operational interpretation of biodiversity protection goals across regulations, (2) limited species representation failing to predict population and ecosystem effects, (3) fragmented assessment approaches, and (4) absent systematic monitoring data integration. Recommendations include revising legal frameworks to enshrine biodiversity as explicit protection goal across all chemical regulations, establishing EU-wide early warning systems linking chemical and biodiversity monitoring data, and integrating biodiversity expertise into regulatory decision-making processes.

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2.12.B.T-04 Occurrence and concentration of organic pesticides in Europe's wildlife

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1. Introduction

Pesticides unintentionally can end up in non-target species and ecosystems where they exert toxic pressure. The environmental fate of pesticides is predicted based on their chemical properties, but this is not systematically monitored after legislation. Therefore it remains unclear if and in what quantities pesticides find their way into terrestrial biota. To shed light on this we systematically collated field data reporting concentrations of pesticides in European wildlife (i.e. vertebrates, invertebrates and plants). We harmonized all data, establish research and monitoring gaps, and analysed the concentration levels of the most common pesticides over time.

2. Materials and Methods

We performed a literature search with which identified data from 190 published publications, yielding 176,257 rows of data of pesticide concentrations in wildlife together with up to 49 columns of metadata. The collected data was curated and integrated into the NORMAN database system. To understand patterns inside the data we calculated the frequency of appearance of chemicals and their respective groupings and analysed the proportional coverage of 257 species and species groups had in monitoring.

3. Results and discussion.

For most chemicals only few data exist with over 300 of 800 pesticides were tested for only once. While over half of chemicals were not studied more than twice. Organochlorines are still the most frequently detected pesticides with an average FoA of 66 % and are the most tested pesticides overall. Other banned pesticides like Carbofuran are also still found decades after ban. The most common compounds that are still legal to use are rodenticides, which also increase in frequency of appearance when compared to data from before 2010. We also identified that most monitoring is performed on key species groups (mammals, birds, crops) with few studies outside that scope. Mammals and birds make up of 50 % of data in almost every pesticide class. Rodenticides for example, report studies exclusively on mammals and birds, except one.

4. Conclusions

We show that regardless of legal status pesticides occur in most wildlife around Europe even after decades of bans. The pollution seems to be persistent, while newer compounds are now also found in wildlife. However, we also show that the breadth of testing is increasing doing the amount of used pesticides justice and allowing a more encompassing look at pesticides in nature.

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2.12.P Terrestrial Biodiversity and Chemical Pollution: Understanding Exposure and Impacts Towards Improving Risk Prevention and Management

2.12.P-Tu140 The Invisible Threat: Tracing Organic Micropollutants Across Diverse European Terrestrial Food Chains Using Cutting-Edge Mass Spectrometry

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Organic micropollutants (OPs) threaten terrestrial ecosystems and biodiversity by causing physiological, reproductive, and immune harm to wildlife, disrupting food webs and ecosystem stability. They biomagnify in food chains, impacting apex predators and causing epigenetic effects that threaten populations over generations. Addressing biodiversity loss from OPs requires integrated policies combining pollution reduction, ecosystem restoration, and strong regulations. Although OPs' impact on aquatic systems is well-studied, terrestrial effects remain under-researched. The EU-funded TerraChem project (2023-2026) fills this gap by monitoring chemical exposure across trophic levels in six European countries using innovative methods. TerraChem's 2024–2025 sampling in the Netherlands, Germany, Romania, Portugal, Spain, and Greece targeted apex predators—beech martens, grey wolves, red foxes, Egyptian mongooses, European badgers, and northern white-breasted hedgehogs—at six sites per country, plus soil, plants, invertebrates, rodents, and prey species. Sample preparation employed optimized methods, including modified QuEChERS for soil/plants and solvent-SPE protocols for tissues, enabling liquid and gas chromatography analyses. Advanced analytical techniques included high-resolution mass spectrometry (HRMS) coupled with liquid chromatography-ion mobility spectrometry (LC-IMS-HRMS), gas chromatography-HRMS (GC-HRMS), and tandem mass spectrometry (GC-MS/MS). GC-MS/MS quantitatively targeted trace levels of priority OPs, while LC and GC HRMS enabled comprehensive targeted and non-targeted screening using a large, curated database maintained by the National and Kapodistrian University of Athens, containing over 3,000 environmentally relevant compounds. Hundreds of OPs were detected, including 18 per- and polyfluoroalkyl substances (PFAS) such as PFOS, PFOA, and 6:2 FTS, concentrated especially in apex predator livers, showing biomagnification. Legacy pesticides like DDT and metabolites persisted despite decades-long bans. Anticoagulant rodenticides (Brodifacoum, Bromadiolone) appeared mainly in livers from Spain, Greece, and Romania. These emerging contaminants and persistent organic pollutants, combined in complex mixtures, act as stressors for wildlife and possibly humans. TerraChem's multi-trophic, state-of-the-art HRMS approach advances knowledge of OP distribution, exposure routes, trophic transfer, and ecological impacts across Europe's terrestrial ecosystems.

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2.12.P-Tu142 Automating Reliable Input Data: The PROVES Python Packages for Chemical Structure and Property Verification in Environmental Modeling

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Characterizing the impact of chemicals on humans and the environment requires several substance-specific thermodynamic, transport, and fate parameters. We implement systematic workflow and computational tools for a transparent and comprehensive evaluation of the most used QSAR models for the prediction of physicochemical and environmental properties of chemical substances required for the USEtox model. The workflow aims to reduce uncertainty and clarify the provenance of parameters, consequently helping to transparently quantify the impact of the input parameters on the USEtox results.

To fully automate this complex workflow, we have developed a family of open-source Python packages collectively known as PROVES (Preprocessing and Verification of Substance Properties). The cornerstone of this suite is PROVESID, a unified library designed for finding chemical identifiers and structures, performing structure verification, and handling conversion between various formats using major public sources, e.g. PubChem, CAS Common Chemistry, and tools e.g. IUPAC OPSIN. It also includes functions for downloading available experimental data. Complementing this, we introduce PyCompTox and PyEPISuite. PyCompTox offers the capability to efficiently search and retrieve properties and data from the CompTox Chemicals Dashboard, while PyEPISuite provides well-structured data access from the EPI Suite web application. Finally, PyQSARToolbox provides an interface to the QSAR Toolbox and its key addons, including OPERA and VEGA, allowing users to programmatically run prediction models and access experimental reference data, making the entire prediction process fully automatable. Finally, EXPROVES provides a collection of literature data for physicochemical and fate properties of chemicals with a Python interface for the verification of the predictions.

Collectively, these PROVES packages streamline the entire process from initial chemical identification and verification to multi-model prediction and systematic quality control. The entire suite of packages will be an integral part of the new USEtox web application and will be released open-source on GitHub under the USEtox organization, providing the environmental modeling community with transparent, traceable, and validated chemical property and environmental data.

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2.12.P-Tu143 A Systematic Workflow for Generating Accurate and Transparent Chemical Input Data for Environmental Modelling

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Environmental models that deal with the transport, fate, and effect of chemicals in a multimedia environment, e.g. USEtox, require reliable data on the physicochemical properties and reactivity of chemical substances. However, due to lack of experimental data, environmental models must rely heavily on predictive methods, often based on Quantitative Structure-Activity/Property Relationships (QSAR/QSPR). Reliance on unverified predictive models can introduce significant uncertainty into environmental risk assessments, potentially leading to inaccurate estimations of chemical impact on human health and ecotoxicity. We address these problems by proposing a new, systematic workflow to improve the transparency and accuracy of USEtox input parameters by systematically verifying predictive performance. We first obtained chemical structures for chemical identifiers. The verified structures are then used in OPERA, VEGA, EPI Suite, and QSAR Toolbox, and IFSQSAR. For each required parameter, we calculate the Root Mean Square Error (RMSE) of the deviations of predicted values from experimental data. We ranked the models by the reducing order of the calculated RMSE and selected the two highest-performing models for each parameter, unless one model significantly outperforms the second best. The systematic evaluation successfully identified the most reliable models: OPERA showed reasonable performance for most physicochemical parameters, although there is still room for improvement for the Henry’s law constant estimation, and almost all models failed to satisfactorily predict acid and base dissociation constants. Notably, EPI Suite showed the best performance for the prediction of degradation rates. This work culminates in the generation of a new transparent database of USEtox input parameters for over 10,000 substances, prioritizing experimental data, the geometric average of the predictions of the two best models, and, lastly, lower-performance model predictions. Each reported value is labeled with full metadata, indicating the source and whether the prediction is within the applicability domain of the utilized model. This open-source workflow and database, which will be available on GitHub, advances environmental modeling by providing a verified and transparent set of input parameters, substantially reducing uncertainty and improving the reliability of chemical impact assessments.

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2.12.P-Tu144 Integrated Solutions for Managing Contaminants of Emerging Concern: TerraChem’s Approach to Data, Modeling, and Visualization in Terrestrial Ecosystems

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Marissa B Kosnik⁶, Martina G. Vijver⁷, S. Henrik Barmentlo⁷, Koos Biesmeijer⁸, Peter Fantke¹, George Gkotsis², Romana Hornek-Gausterer⁹, Olivier Jolliet¹⁰, Oliver Machate⁵, Peter Oswald¹, Laura Scherer⁷ and Jaroslav Slobodnik¹, (1)Environmental Institute s.r.o., Slovakia, (2)National and Kapodistrian University of Athens, Greece, (3)Naturalis Biodiversity Center, Slovakia, (4)Technical University of Denmark (DTU), Denmark, (5)German Environment Agency (UBA), Germany, (6)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (7)Leiden University, Netherlands, (8)Naturalis Biodiversity Center, Netherlands, (9)Technikum Wien, Austria, (10)Danish Technical University, Denmark

TerraChem establishes an integrated framework for identifying contaminants of emerging concern (CECs) in terrestrial ecosystems by combining novel sampling approaches, advanced high-resolution mass spectrometric (HRMS) analysis, FAIR data management, harmonized data processing, and predictive modelling. The TerraChem Data Management System (TDMS) structures and disseminates all project information. It includes a comprehensive sample catalogue containing rich metadata on collected specimens; a chemical occurrence database storing targeted monitoring results; a literature-derived occurrence database mined from scientific publications; and the Digital Sample Freezing Platform (DSFP), which stores and processes untargeted HRMS data and performs wide-scope suspect screening across ~95,000 contaminants included in the Substance Database. All modules expose data through open APIs. A new module, the TerraChem Early Warning System (TEWS) processes DSFP outputs filtered for terrestrial biota, grouping HRMS features and modelling their spatial distributions, temporal behaviours, and biomagnification potential using generalized additive models. A Priority Index is derived to highlight unknown or insufficiently characterized compounds that display hotspots, historical trends, or complex bioaccumulation patterns. Once identities are elucidated, these early signals feed into the broader PARC Early Warning System, which applies hazard, exposure, and ecotoxicological modelling to classify chemicals as NERCs or non-NERCs.

Disclaimer/Disclosure:

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2.12.P-Tu145 Spatially-explicit Impact Assessment of Pesticide-induced Terrestrial Biodiversity Loss yuyue zhang¹, Susan Oginah¹, Farshad Soheilifard¹, Peter Fantke² and **Olivier Jolliet¹**, (1)Technical University of Denmark (DTU), Denmark, (2)substitute ApS, Denmark

Chemical pesticides exert pressure on terrestrial ecosystems through ecotoxicity effects on organisms. Global reduction targets, including the UN Sustainable Development Goals, call for decreased pesticide use, yet a spatially-explicit global overview of impacts on terrestrial biodiversity is lacking. Existing studies focus on specific regions or extrapolate from limited data, leaving no overview that accounts for regional differences in species vulnerability. Biodiversity hotspots with endemic species face disproportionately higher extinction risks, yet current Life Cycle Impact Assessment methods lack the spatial resolution to capture this variation.

To address this gap, we integrate the full impact pathway from pesticide use through emissions and environmental fate to damage on terrestrial species. Three objectives are addressed: (1) Linking pesticide application to Predicted Environmental Concentrations (PEC) in soils using the multimedia Pangea fate model. (2) Combining PEC with Global Extinction Probability (GEP) data for terrestrial taxonomic groups to calculate area-normalized impact scores. (3) Identifying terrestrial biodiversity impact hotspots to inform prioritization for pesticide reduction.

Spatially-resolved emissions were modeled based on application method, crop type, and growth stage. Pangea estimated PEC for glyphosate across 87,063 global catchments. Biodiversity impact was calculated as: $\text{Damage} = \text{PEC} \times \text{EF} \times \text{XF} \times \text{GEP}$, expressed as Potentially Disappeared Fraction (PDF) per year per km².

Results show highest impact intensities where intensive agricultural use overlaps with high-GEP regions, particularly in Brazil, Argentina, Central Europe, India, and China. Some high-GEP regions (e.g., Congo Basin) show lower damage due to limited pesticide application, while moderate-GEP regions (e.g., US Midwest) show elevated damage due to intensive use. This framework enables identification of impact hotspots and supports prioritization of interventions in biodiversity-sensitive areas.

2.12.P-Tu146 Assessing the Temporal and Spatial Distribution of Chemical Exposure via Heavy Metal and Other Element Concentrations in Peregrine Falcon Down Feathers in the Netherlands Using INAA **Cristina Ruiz González¹**, Paola Movalli², Lambert van Eijck³, Peter Bode³, Mehmet Sarilar³ and Delia van Rij³,

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Understanding chemical exposure in terrestrial wildlife is essential for detecting environmental and human health risks, providing early warnings of emerging contaminants and improving our knowledge of how pollutants affect European biodiversity. As part of this broader effort, this study assesses the temporal and spatial distribution of heavy metals and other elements in peregrine falcon (*Falco peregrinus*) feathers in the Netherlands, contributing both to EU priorities on addressing chemical risks and to TerraChem's objective of improving understanding of chemical stressors in terrestrial biota. A statistically valid number of chick down feathers from the Naturalis Biodiversity Center, collected in five different years between 2017 and 2024, were analysed to establish historical reference values for elements of societal concern. These baselines support interpretation of current concentrations and spatial patterns derived from recent (2024) samples collected across more than 50 nest locations, including Eindhoven, Veldhoven, Duiven, 's-Hertogenbosch, Budel, Apeldoorn, Maastricht, Zwolle, Arnhem and Meppel, among others. The sampled sites represent urban, industrial and rural areas, allowing exploration of spatial patterns linked to potential pollutant sources. All feather samples were analysed using instrumental neutron activation analysis (INAA), a non-destructive technique that provides consistent quantitative data across years and sites and allows examination of concentration values to compare historical and more recent exposure patterns. The resulting dataset will offer reference values for selected contaminants in peregrine feathers, supporting more accurate interpretation of current exposure levels and revealing trends in elements that may pose risks to peregrines, wider terrestrial ecosystems or human health. The final results and conclusions will be presented in the poster.

2.12.P-Tu147 Do Gammarids Play a Role in the Transmission of Anticoagulant Rodenticides?

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Tracking the environmental fate of anticoagulant rodenticides (AR) across food webs can be challenging. Aquatic exposure pathways of these problematic substances appear to be more diffuse than terrestrial, suggesting multiple sources of contamination at trace level. One understudied group of organisms in terms of rodenticide exposure and transmission are invertebrates, which serve as a nutrition base for a variety of higher tier species. In the present study, the abundant amphipod crustacean *Gammarus* sp. was chosen as a model organism for freshwater ecosystems to investigate two hypothetical exposure pathways. Although known as shredders, gammarids are opportunistic omnivores, and thus potentially attracted to baits or poisoned carcasses flushed into surface waters during rodent baiting campaigns. Here, we studied the behavior of freshwater gammarids (*Gammarus pulex* and *G. roeselii*) feeding on commercial rodenticide bait blocks under controlled laboratory conditions. In a second laboratory experiment, gammarids were offered poisoned rodent liver tissue as a food source. Rodenticides comprised the second-generation active ingredients brodifacoum and bromadiolone. In each experiment, we measured the AR body burden of gammarids after 36 hours of exposure using LC-MS/MS. In addition, a field monitoring was conducted, and native freshwater gammarids collected from 25 German surface water locations were screened for AR residues.

Overall, our results show that gammarids consumed offered bait as well as rodent liver tissue in the laboratory experiments. The AR body burdens of gammarids exposed to bait were in the range of 0.72 µg/g dry weight (bromadiolone) and 1.65 µg/g dry weight (brodifacoum), respectively. Residue levels were below the method quantification limit of 0.015 µg/g dry weight in controls feeding on alder leaves. Dietary uptake of fluorescent bait by gammarids was visually confirmed using a stereomicroscope. No mortality associated with rodenticide bait or poisoned tissue consumption was observed over a 36 hours exposure period. Unlike vertebrates, invertebrates are not susceptible to the specific mode of action of AR. All of the randomly collected 25 field monitoring samples revealed no quantifiable AR residues. Yet, our straightforward laboratory experiments demonstrate that gammarids may contribute to the transmission of AR in the aquatic food web after their opportunistic feeding on bait or poisoned carcasses.

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soil water contaminations for plants, and to aerial transport of pesticides for aphids. This study thus showed that pesticide exposures within non-target ecosystems adjacent to crops drive, at least to short-term, organisms' responses to later exposure to sublethal doses of pesticides, with different outcomes depending on the considered organism.

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2.12.P-Tu150 Chemicals in Agricultural Soils in Germany

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The aim of the project 'Chemicals in Agricultural Soils in Germany' is to provide a first overview on the contamination of agricultural sites with different types of chemical pollutants. Soil samples were collected in the years 2022 – 2024 from 110 sites across Germany, covering arable land (62), grassland (30), orchards (9) and vineyards (9). The chemical monitoring focused in a first step on a comprehensive screening of pesticides, i.e. active substances and metabolites of plant protection products and biocides, pharmaceuticals and a small number of selected industrial chemicals in two soil layers (0–5 cm and 5–20 cm). Within a second step, a subset of substances was analysed with lower detection limits. In addition, neonicotinoids were analysed separately with significantly lower detection limits. Concentrations of the analytes were on average lower in the deeper soil layer. Insecticides, particularly imidacloprid and clothianidin, were the most frequently detected compounds across all sites. The diversity and total number of detected substances varied considerably between locations, but no clear geographical pattern was observed. Arable soils showed the highest prevalence of fungicides, herbicides and insecticides. Pharmaceuticals and industrial chemicals were only rarely detected.

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2.12.P-Tu151 Cumulative Risk in Soil

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This study provides an overview of pesticide (plant protection products, biocides and metabolites) contamination in agricultural soils in Germany, measured at 110 sites sampled between 2022 and 2024 across arable land, grassland, orchards, and vineyards. Measured concentrations of active substances were evaluated using ecotoxicological threshold values to characterize potential risks for soil organisms. Toxic Units (TUs) were calculated for each detected compound, using Regulatory Acceptable Concentrations (RACs) for plant

protection products (PPPs) derived from data for soil invertebrates and Predicted No-Effect Concentrations for soil (PNECsoil) for biocides. Cumulative Risk Indicators (RIs) were determined by summing all substance-specific TUs per site, applying the principle of concentration addition to capturing mixture effects. PPP-related TUs showed that a few soils exhibited exposure levels of ecological concern. Six arable fields exceeded RI values above 1, indicating that acceptable thresholds were surpassed. Vineyards and orchards also showed RIs > 1, though generally at fewer sites. In contrast, grassland displayed consistently low risk index values. Key risk drivers included Dimoxystrobin, Boscalid and Clothianidin, as well as isolated exceedances from Hydroxysimazine. Mixture risks in high-impact sites were typically dominated by one or two substances. Biocide contamination contributed less overall but still produced notable risk at individual sites. PNEC-based TUs revealed exceedances for Clothianidin, Fludioxonil, and Azoxystrobin, with highest cumulative biocide RIs observed in arable soils. However, these substances could have been used as plant protection products on arable fields. The only biocides found which are not also authorized as plant protection products, the quaternary ammonium compounds (BAC12, BAC14), both generally played a minor role within the overall RI. Overall, the results show that cumulative chemical exposure in agricultural soils may adversely affect soil invertebrates, and that combined PPP and biocide mixtures could exert additive effects not captured through single-substance evaluation. The study underscores the need for continued chemical and biological soil monitoring, inclusion of monitoring results in the risk assessment, improved mixture-risk assessment approaches, and consideration of cumulative chemical pressure in regulatory frameworks.

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2.12.P-Tu152 Earthworm Communities in German Agricultural Soils: Patterns, Drivers and Chemical Risk

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The aim of the project ‘Chemicals in Agricultural Soils in Germany’ is to provide a first overview on the contamination of 110 agricultural sites with different types of chemical pollutants. In addition to the chemical monitoring, the earthworm communities were analysed at 51 sites representing arable land, grassland, orchards, and vineyards. On average, total earthworm abundance and biomass were highest in grassland and lowest in arable land and vineyards reflecting the influence of land use and management intensity. However, variability within the arable soils was high - the highest abundance and biomass values were found here. Multivariate analyses revealed that soil properties (e.g., pH, organic carbon, texture) were the primary drivers of earthworm abundance and biomass, while cumulative chemical risk indicators also negatively influenced earthworm abundance and biomass. These findings highlight the complexity of soil ecosystems and the need for integrated monitoring approaches that consider both chemical mixtures and ecological context.

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2.12.P-Tu153 Tropical Mesocarnivores as Heavy Metal Sentinels in Proximity to Oil Palm Plantations

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The Bornean state of Sabah has lost ~40% of its forests to oil palm. Despite this, generalist mesocarnivores such as civets and leopard cats persist in plantations, potentially increasing exposure to inorganic pollutants, including heavy metals from agrochemicals and regular chemical fertiliser use. We assessed acute and chronic non-essential heavy metal burdens in wild mesocarnivores from the Lower Kinabatangan Wildlife Sanctuary, where remnant forest lots are surrounded by plantations, using inductively coupled plasma optical emissions spectroscopy (ICP-OES) of blood and hair samples. Blood samples were used to provide acute estimates of exposure, and hair samples were used to estimate chronic exposure.

Sunda leopard cats, as a model species for a persistent plantation predator, show elevated arsenic (As) (acute: 9.86 ± 1.56 ; chronic: 2.86 ± 2.65 mg/kg) and lead (Pb) (0.32 ± 0.31 ; 1.93 ± 3.15 mg/kg). Common palm civets, as generalists with a preference towards frugivory, exhibit elevated As (0.95 ± 0.70 ; 1.11 ± 0.60 mg/kg), Pb (0.74 ± 0.33 ; 0.80 ± 0.53 mg/kg), and aluminium (Al) (43.43 ± 23.92 ; 24.56 ± 13.43 mg/kg). Malay civets as generalists show high Al (38.94 ± 25.02 ; 53.16 ± 46.83 mg/kg), As (0.95 ± 0.33 ; 1.10 ± 0.19 mg/kg), cadmium (Cd) (0.41 ± 0.79 ; 0.12 ± 0.07 mg/kg), mercury (Hg) (2.69 ± 5.64 ; 7.88 ± 12.82 mg/kg) and Pb (0.98 ± 0.63 ; 0.55 ± 0.29 mg/kg).

Results suggest that metal bioaccumulation is a constant pressure, with As and Pb particularly elevated in plantation-persistent mesocarnivores and those living in proximity to plantations. All elevated non-essential metals exceeded thresholds of concern for each study species, raising urgent questions about the future for mesocarnivore persistence in oil palm systems and the invisible impacts of extensive agriculture on tropical communities.

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2.12.P-Tu154 Nano-Enhanced Phytoremediation of Cd-Contaminated Soil: ZnO NPs Stimulate Brassica juncea Growth and Stress Tolerance

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Heavy metal (HM) contamination remains a persistent environmental challenge, as these elements do not degrade and accumulate in soils, plants, and food webs. Phytoremediation provides a sustainable approach for HM removal, but its efficiency is often limited by slow plant growth and metal-induced stress. In parallel, engineered nanomaterials—particularly zinc-based formulations—are increasingly used in agriculture to enhance nutrient availability, strengthen stress tolerance, and stimulate biomass production. Their reactivity has recently prompted interest in applying them as soil amendments to support remediation, yet nano-assisted phytoremediation remains at an early stage, with major gaps concerning nanoparticle–metal interactions, mixture effects, and safe but effective dose ranges.

To address these questions, we evaluated how different concentrations of ZnO nanomaterial (0, 100, 200, 300 mg kg⁻¹) influence Indian mustard (*Brassica juncea*) grown in Cd-contaminated soils (0–100 mg kg⁻¹) under controlled ambient conditions (21/14°C day/night; 400 ppm CO₂; 55–70% RH). Plant responses were assessed via chlorophyll content, shoot height, root length, and shoot and root dry biomass, representing key indicators of physiological stress and phytoremediation performance.

Cadmium reduced all growth traits in a concentration-dependent manner. In contrast, low-dose Zn NPs amendments partially alleviated Cd stress, increasing chlorophyll retention, promoting root elongation, and enhancing shoot and root biomass by ~10–30% relative to Cd-only controls. The strongest improvements occurred at 1–10 mg kg⁻¹ Cd, likely reflecting micronutrient supplementation and reduced Cd adverse effects. At 50 mg kg⁻¹ Cd, mitigation effects remained but were insufficient to fully counteract toxicity, whereas the highest nanomaterial dose (300 mg kg⁻¹) occasionally intensified HM stress, indicating a potential shift from beneficial to inhibitory effects.

Overall, the findings show that small additions of ZnO NPs amendments can modulate plant stress responses, enhance growth, and increase Cd accumulation, thereby improving the overall phytoremediation efficiency of

contaminated soils. These results provide new insight into mixed nanoparticle–HM interactions and underscore the promise—and the need for careful dose optimisation—of nano-assisted phytoremediation in environmental risk management.

2.12.P-Tu155 Unraveling Soil Interactions and Ecological Risks of Zinc Oxide Nanoparticles and Cadmium

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Global population growth is expected to reach 9.6 billion by 2050, intensifying pressure on agricultural soils and increasing the need to manage contamination while sustaining productivity. Nanotechnology has emerged as a promising tool to enhance crop performance and support heavy metal (HM) remediation. Due to their high surface area and reactivity, nanoparticles (NPs) can reduce metal toxicity or stimulate plant growth at low doses, yet excessive inputs may create new risks, as unabsorbed particles can persist in soil, alter physicochemical properties, and affect soil biota. When applied to contaminated soils, NPs may also modify soil functioning in ways that influence both plant performance and ecological safety, underscoring the importance of evaluating combined HM–NPs effects.

To investigate these interactions, we conducted a phytoremediation experiment with *Brassica juncea* grown at 21°C in soils amended with cadmium (0, 1, 10, 50 mg/kg) and ZnO nanoparticles (0, 0.1, 0.2, 0.3 g/kg). Soil CO₂ and O₂ concentrations, electrical conductivity (EC), soil water content (SWC), pH, temperature, and organic matter were monitored to assess soil–plant–microbe responses. Moderate ZnO additions (0.1–0.2 g/kg) enhanced soil respiration, increasing CO₂ from 10% to 2.8-fold above untreated soil, suggesting stimulated microbial and root activity. O₂ decreased slightly (~1–8.2%), consistent with elevated respiration, with stronger declines as Cd increased from 0 to 10 mg kg⁻¹. EC and SWC remained stable at moderate NP doses but declined at 0.3 g kg⁻¹, indicating potential stress on soil structure or biota. Soil pH varied only ±0.3 units around neutrality. Organic matter increased markedly across all Cd–ZnO treatments, rising by 4.4–70.4% relative to the control without ZnO NPs.

Overall, the study shows that ZnO nanomaterials have a dual role in Cd-contaminated soils: low to moderate doses enhance biological activity, support Cd stabilization, and improve conditions relevant to phytoremediation, whereas excessive NP inputs or elevated Cd levels reduce soil respiration and disrupt moisture dynamics. These findings advance the ecological risk assessment of HM–NP mixtures and highlight the need for careful dose optimisation to enable sustainable nano-assisted bioremediation strategies that balance remediation efficiency with ecological safety and agricultural productivity.

2.12.P-Tu156 Removal of emerging contaminants in wastewater treatment: comparison of photosynthetic systems and activated sludge

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Contaminants of emerging concern (CECs) are chemical substances that are commonly detected in the environment and can cause ecological or human health effects. In this scenario, algal-bacterial processes have recently emerged as an economically viable and environmentally friendly option for eliminating chemical and biological contamination from different types of wastewaters: domestic, industrial and agricultural. Utilizing solar energy, wastewater treatment with microalgae converts carbon and nutrients into algal and bacterial biomass and showed similar high removal efficiencies with membrane bioreactor of activated sludge systems.

2.12.P-Tu157 Ecotoxicological Dynamics of Insecticide Resistance in Cotton-Feeding *Spodoptera littoralis*

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The cotton leafworm (*Spodoptera littoralis*) (Boisduval) (Lepidoptera: Noctuidae), is one of the primary pests in cotton production, causing significant yield losses due to its high population densities. This situation has led to increased pesticide applications, resulting in the development of insecticide resistance in the pest. Effective pest management today requires determining the resistance status to insecticides and investigating the effects of synergistic substances on resistance mechanisms. In this study, the resistance of *S. littoralis* populations collected from the Aydın region of Turkey to active ingredient indoxacarb was examined. Additionally, the effects of commonly used synergists diethyl maleate, piperonyl butoxide, and triphenyl phosphate on the resistance mechanisms of the pest to insecticides were evaluated.

Experiments were conducted following the methodology recommended by the Insecticide Resistance Action

Committee (IRAC). Second instar larvae were exposed to six different doses with three replicates placed in petri dishes, each containing 10 larvae. Mortality was recorded at 24 and 48 hours, and LC50 values were calculated using the Polo Plus program to assess resistance levels. To evaluate the effects of synergistic substances, 1 µl of each synergist was applied to third instar larvae using a Hamilton syringe, and mortality rates were recorded after 24 and 48 hours.

The results indicated that the pest population has developed insecticide resistance, while synergistic substances played a significant role in overcoming resistance mechanisms. These findings allow for the optimization of pesticide applications by selecting effective insecticides and utilizing synergists in resistance management. Consequently, unnecessary pesticide use can be reduced, promoting sustainable pest control. Future studies are planned to investigate resistance mechanisms at the molecular level and explore new synergistic combinations.

2.13.A Unpacking Plastic Complexity: Investigating the Biological Impacts of Micro- and Nanoplastics, and Plastic Associated Chemicals

2.13.A.T-01 A New Methodology for the Quality Assessment of Recycled Plastics

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To establish wide spread recycling of plastic in the packaging and other short-life segments it is necessary to monitor the quality of the materials with methods that appropriately address their complexity while being practical in day-to-day operations. A recyclates quality is determined based on the release of potentially harmful substances in the context of this study. The challenge lies in the large variety of possible substances ranging from additives, degradation products, non-intentionally added substances to all kinds of contaminants and the realistic description of their behaviour. While this situation calls for comprehensive analysis, the volume based low-cost business model of plastic packaging and by extension also its recycling call for fast decisions based on practical parameters.

As basis for the method development a migration scenario at room temperature and 70°C utilizing water as eluent was devised and implemented with recycled polypropylene pellets from different input streams. The produced eluate was assessed in terms of released dissolved organic carbon (DOC) and estrogenic potential. First results show the influence of an increased temperature during elution on the DOC value which appears to be connected to the release of compounds with estrogenic potential for one sample only. To complement these tests with data about the release of individual substances passive sampler mediated thermal desorption gas chromatography mass spectrometry (TD-GCMS) is used in the eluates. On the level of single substances, the differences between samples become less obvious but an evaluation scheme based around congruent and unique signals opens a new angle at the problem. By dividing signals from the TD-GCMS analysis in this manner two goals can be achieved. Firstly, insights about the commonly found substances in these recyclates are generated and secondly it acts as a filter to focus on relevant signals likely associated with effects observed in individual samples. Finally, a database storing all signals from past measurements attributed to their respective samples is continuously maintained. With this approach each additional processed sample will improve the clarity of correlations between signals, observed effects and input materials used for recycling.

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2.13.A.T-02 Intestinal Toxicity of Polyethylene Terephthalate Micro- and Nanoplastics and Their Degradation Products: A Multi-Endpoint Cellular Analysis

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The increase in plastic production has generated large amounts of waste, much of which enters in the environment and degrades into small particles, classified as microplastics (<5 mm) and nanoplastics (<100 nm) (MNPs). The widespread of MNPs in the environmental resulted in their recognition as emerging pollutants, raising the risk of human exposure to both the particles and their degradation products (DPs). Ingestion is considered the primary exposure route, allowing both MNPs and DP to interact with gut epithelium. However, their cellular and molecular effects remain largely unknown. Understanding the cytotoxic and mechanistic

effects of MNPs and their degradation products is essential to assess their potential risks to human health. Polyethylene terephthalate (PET), a plastic widely used in food and beverage packaging, can be degraded into bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate (MHET), terephthalic acid (TPA) and ethylene glycol (EG). Here, the intestinal cell line Caco-2 was used to evaluate the effects of PET particles at micro and nanosize (12µm;80nm) and PET-degradation products (BHET; MHET; TPA; EG) on cell viability, ROS production, cell-cycle dynamics and apoptosis. PET particles induced a slight decrease in cell viability and no detectable effects on cell-cycle dynamics or apoptosis. In contrast, PET-DP showed a more pronounced biological activity. MHET showed a dose-dependent cytotoxic effect and increased the ROS production, indicating oxidative stress despite the absence of cell-cycle or apoptotic alterations. Similarly, BHET reduced the cell viability, and induced the cell cycle arrest at G2 phase and early apoptosis. TPA did not cause changes in none of the endpoints tested, whereas EG produced no cytotoxic or oxidative effects but decreased the percentage of cells at G2 phase and increased the percentage of cells undergoing late apoptosis, suggesting more subtle modulatory actions. Together, these findings demonstrate that PET particles have relatively low adverse effects in intestinal cells, while PET-DP, particularly MHET and BHET, showed a more intense cytotoxic and mechanistically effects. These results highlight the importance of hazard assessments for both MNPs and its by-products to a more accurate evaluation of the potential health risks associated with human exposure.

Disclaimer/Disclosure:

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2.13.A.T-03 Breaking Ground: The Effect of Leachates from Conventional and Biodegradable Mulching Films on Plants

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The growing use of plastics in agriculture, such as mulching films, has led to the accumulation of plastic residues in soils. Biodegradable plastics are often proposed as an alternative to conventional plastics because of their presumed faster degradation rates. However, both types are subject to weathering processes and can release degradation products into the environment. Despite this, the effects on plant development of these leached chemicals and residues remain poorly understood. This study investigates the impact of artificially weathered plastic leachates on four widely cultivated crops: two monocots, barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), and two dicots, carrot (*Daucus carota*) and lettuce (*Lactuca sativa*). We compared conventional low linear density polyethylene with a biodegradable starch-polybutylene adipate-terephthalate blend. Effects on plant growth were assessed during germination and early development, as well as in a chronic pot experiment (lettuce and barley only), using concentrations of 0.01%, 0.1%, and 1% w/w. The acute exposure showed limited effects overall, with root length being the most sensitive endpoint for both monocots and dicots. Interestingly, an increase in root length was observed for both barley and lettuce. During chronic exposure, the increase in root length persisted for these species. In addition, lettuce seedlings showed a decrease in shoot fresh weight. Importantly, the plant defence mechanisms were upregulated in both species for both plastic types, indicating that the exposure to leachates causes stress to plants. Our work is among the first to directly compare the effects of leachates from conventional and biodegradable plastics on plants. The study demonstrates that the impact of mulching film residues is not solely a physical issue (i.e., fragment presence) but also a chemical one, via leached compounds. Although the immediate impact of conventional and biodegradable plastic leachates appeared limited, they nonetheless produced measurable adverse effects that could be amplified under natural and multi-stressor conditions.

Disclaimer/Disclosure:

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2.13.A.T-04 Multi-Species Toxicity of Leachates Containing Microplastics, Nanoplastics and Additives from Artificially Aged Oil-Based and Partially Biobased and Biodegradable Fishing Nets

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Plastic gillnets represent one of the most extensively used and lost fishing gear worldwide. However, their contribution to the microplastic (MP), nanoplastic (NP) and chemical pollution of the ocean is still insufficiently understood. To fill this knowledge gap, one partially biobased and biodegradable gillnet made of polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT) and three gillnets made of polyamide (PA) were artificially aged (25 days at 23°C) using UV irradiation (UV-A and UV-B) and then agitation (24h at 190 rpm) with abrasion (glass microbeads) in water. Leachates of non-aged gillnets were also produced using the same protocol. The toxicity of the leachates produced was evaluated in four aquatic organisms (*Aliivibrio fischeri*, *Tisochrysis lutea*, adult *Acartia tonsa*, fish *Oryzias latipes* larvae). All the studied gillnets produced MPs (median size 23.6 µm, with a shape of fragment) and NPs. On average, the non-aged and aged PA gillnets produced 4.1x10⁴ MP per gram of FN (95% CI: 3.5x10³-6.6x10⁴) and 3.1x10⁵ MP per gram of FN (95% CI: 8.4x10³-6.6x10⁵), respectively. In comparison, the PBS-PBAT gillnet produced less MPs with 2.7x10⁴ MP per gram of FN (95% CI: 1.5x10³-6.8x10⁴) and 9.7x10³ MP per gram of FN (95% CI: 2.2x10⁻⁴-2.8x10⁴), respectively. Four trace metals were quantified in the leachates (Cu, Fe, Pb, Zn). Moreover, 7 organic plastic chemicals were quantified in the leachates, some of which are known to be hazardous to aquatic species. Three leachates of non-aged FNs (2 PA and the PBS-PBAT) and leachates of one aged FN (PBS-PBAT) caused significant *A. fischeri*'s bioluminescence inhibition (16% to 23%). None of the leachates caused a significant growth inhibition of *T. lutea*. Leachates of two FNs (one aged PA and the aged and non-aged PBS-PBAT) caused significant mortality in *A. tonsa* (37.8±13.3% to 41.1±11.3%). The three PA FNs significantly disrupted the heartbeat (± 13% to 18%) and behavior (behavior stress index (BSI): 0.5 to 7) of *O. latipes*' larvae. The PBS-PBAT FN only affected the behavior of the fish larvae (BSI: 1 to 1.5). Based on PCA results, the observed toxicity was predominantly attributed to the plastic chemicals released. Taking into account the 4 species tested, the PBS-PBAT FN displayed a similar toxicity than the oil-based PA gillnets. The findings presented herein indicate that rigorous toxicity assessments are imperative prior to the introduction of novel biobased and biodegradable nets into the market.

Disclaimer/Disclosure:

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2.13.B Unpacking Plastic Complexity: Investigating the Biological Impacts of Micro- and Nanoplastics, and Plastic Associated Chemicals

2.13.B.T-01 Plastic Litter in the Arctic as a Research Asset: Investigating the Impacts of environmental Nanoplastics and Associated Contaminants on Human Digestive Health

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Plastic pollution has reached the most remote regions on Earth, including the Arctic. Particulate plastics have been detected in various media of this fragile ecosystem, making exposure for Arctic biota unavoidable. Given that coastal Arctic communities rely heavily on a traditional marine-based diet, the presence of micro- and more

recently nanoplastics (NPLs) raises concerns about potential health risks for both wildlife and humans even in these supposedly “pristine” regions. However, the impacts of environmental NPLs on the first biological barrier they encounter after oral exposure, the human digestive tract, remain largely unknown. To address this knowledge gap, we use an innovative NPL model produced through mechanical extraction of weathered plastic surfaces collected directly from Arctic shorelines [1]. First batches generated from polystyrene debris collected in partnership with local communities from Northern Iceland, demonstrate the feasibility of generating environmentally relevant random-shaped NPLs within the desired size range (<300 nm), suitable for biological toxicity assessments. Plastic debris should not be viewed as inert materials; they can carry a complex mixture of chemicals, including environmental contaminants, notably persistent organic pollutants. Among these, PFAS, and in particular PFOA, have been detected in various Arctic media, and are known to adsorb onto plastic debris [2]. Using human intestinal in vitro models, we assess the toxicity of these emerging contaminants, alone and potentially adsorbed onto NPLs (the “Trojan horse” effect). Experiments using Caco-2 monolayers revealed a dose-dependent reduction in transepithelial electrical resistance following repeated PFOA exposure, suggesting impaired barrier integrity, which was attributed to cell death. The subcellular distribution of PFOA was visualised using high-resolution FIB-SIMS imaging in cell cytosol. By coupling environmentally derived nanoplastics with Arctic-relevant contaminant profiles, this study advances current models of ingestion-based plastic toxicity and provides evidence crucial for public health guidance and policy planning in Northern communities.

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[2] AMAP Assessment 2016: Chemicals of Emerging Arctic Concern., Arctic Monitoring and Assessment (2017)

2.13.B.T-02 Organelle crosstalk as a central regulator of micro- and nanoplastics-induced stress responses: an in vitro approach

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Human exposure to micro- and nanoplastics (MNPs) is increasing, yet their complex composition complicates the prediction of biological effects. To identify mechanistic patterns that may recur across particles and biological systems, we examined how selected engineered nanoparticles, PS beads, digestion-relevant LDPE microplastics, and virgin or UV-aged MNPs influence cellular stress responses in developmental (hDPSCs) and intestinal (co- and tricultures) in vitro models. Analyses included cytotoxicity, redox homeostasis, mitochondrial morphology, cell cycle dynamics, and gene expression of ER stress, mitochondrial dynamics, autophagy, and ferroptosis markers. Not all particles were tested in all models; therefore, the observations reflect mechanistic tendencies rather than direct comparison between models/particles.

In hDPSCs, Ag, TiO₂, and PS nanoparticles activated distinct stress pathways linked to their chemical reactivity and surface properties. Ag nanoparticles induced pronounced lipid peroxidation, ferroptotic signaling, and disruption of mitochondrial dynamics, driving dose- and time-dependent cytotoxicity and altered cell cycle progression. PS nanoparticles induced limited cytotoxicity but exhibited signatures of ER- and lysosomal-associated stress, consistent with altered autophagy and ion imbalance effects. TiO₂ nanoparticles were non-cytotoxic and elicited only mild ER stress and subtle mitochondrial remodeling while supporting osteogenic differentiation. All nanoparticles are localized primarily within endosomal compartments.

In intestinal epithelial systems, virgin and UV-aged PA, PET, PVC, and PS microplastics caused minimal barrier disruption but produced early transcriptional suppression of genes related to ER stress, antioxidative defense, autophagy, and ferroptosis, followed by later compensatory activation. In a separate context, stomach-acid-weathered LDPE generated enhanced extracellular ROS due to surface and compositional changes, initiating marked mitochondrial fusion and branching in Caco-2 cells even without measurable particle uptake. These findings across distinct experimental settings indicate that diverse particles can engage stress pathways centered on mitochondria-ER cross-talk, involving ER stress, redox imbalance, autophagy, and mitochondrial remodeling. These shared mechanistic themes may help guide future work aimed at resolving the sequence and hierarchy of signaling events underlying particle-induced cellular stress.

2.13.B.T-03 Microplastic Fiber-Induced Transgenerational Epigenetic Disruption Impairs Fitness in *Daphnia magna*

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Microplastic fibers (MPFs) are the most prevalent type of microplastics in freshwater environments. However, although the effects of plastic pollution on various aquatic species have been extensively studied, its intergenerational impact on aquatic organisms remains largely unknown. Here, we investigated the multi and transgenerational toxicity of MPFs in *Daphnia magna* over three generations. Our findings revealed that MPF exposure negatively affects reproduction and molting frequency across generations. Notably, the transgenerational groups exhibited reproductive impairments and reduced molting frequency in the F1 and F2 generations (F1T and F2T), despite never being directly exposed to MPFs. Furthermore, whole-genome bisulfite sequencing revealed that these transgenerational adverse effects are associated with differential DNA methylation patterns in specific genes, suggesting that MPFs may threaten *Daphnia* populations through epigenetic modifications. Collectively, our findings provide valuable insights into the intergenerational toxicity of MPFs and the underlying molecular mechanisms driving these responses.

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2.13.B.T-04 Behavioural and Sub-lethal Effects of Microplastic Exposure in Key Coastal

Macroinvertebrates

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Microplastics pose a serious threat to marine and coastal ecosystems, yet their behavioural and physiological effects on macroinvertebrates remain poorly understood. This study investigated the sub-lethal effects of polypropylene and polystyrene microplastics on two ecologically significant coastal species: sandhoppers (Talitridae) and brown kelp beetles (Tenebrionidae). Specimens were exposed to three environmentally relevant microplastic concentrations (0.25, 0.50, and 1.00 mg g⁻¹ of sediment) and a control across four exposure durations (1, 7, 14, and 21 days) under controlled laboratory conditions. Behavioural endpoints, including flipping time, latency to first jump, jump frequency, jump height, burrowing time, distance moved, and velocity, were quantified using live filming and EthoVision XT software. Ingested microplastics were recovered from macroinvertebrates using a soft digestion protocol, enabling precise quantification and characterisation of internalised particles. This study provides robust evidence of sublethal behavioural alterations, emphasising the vulnerability of beach fauna to plastic contamination. These results highlight the ecological risks posed by pervasive plastic pollution in coastal environments.

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2.13.P Unpacking Plastic Complexity: Investigating the Biological Impacts of Micro- and Nanoplastics, and Plastic Associated Chemicals

2.13.P-We141 Effects of Leachate Prepared from Plastic Bag Microplastics on Aquatic and Terrestrial Organisms

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Plastic pollution is a major global concern, with plastic bags contributing significantly to environmental waste. To mitigate this the use of biodegradable alternatives such as polybutylene adipate terephthalate (PBAT) and polylactic acid (PLA) is promoted. However, recent studies indicate that these materials can fragment under environmental conditions, releasing microplastics (MP) and potentially harmful chemical additives. Data on additive composition, leaching behavior, and ecological effects of biodegradable plastics remain scarce. This study assessed leachates from three bag types: conventional polyethylene (M1) and two certified biodegradable starch-PBAT blends: home-compostable (M2) and industrially compostable (M3) bags. MPs were prepared by cryomilling the bags and characterized via imaging techniques. Leaching was performed under high-exposure conditions (5 g MPs/L) in OECD TG 202 medium for 14 days at 37°C with continuous stirring. Filtrates were analyzed for particle presence (SEM) and additive content. Ecotoxicity of leachates (1% v/v) was evaluated on aquatic species (waterflea *Daphnia magna*, duckweed *Lemna minor*, protozoan *Tetrahymena thermophila*) and terrestrial plants (buckwheat *Fagopyrum esculentum*, *F. tataricum*) using established protocols. Endpoints included growth, chlorophyll content, photochemical efficiency, transpiration, and viability. Microplastic size varied significantly among materials (mean $\pm$ SD: M1 = 629.5 $\pm$ 537.4 μ m; M2 = 872.5 $\pm$ 613.4 μ m; M3 = 990.7 $\pm$ 635.9 μ m; $p < 0.001$). No particles were detected in filtrates. Additive analysis revealed irgafos 168 (394 μ g/g) in M1, while M3 contained triethyl acetyl citrate (14 μ g/g) and irganox 1010 (13 μ g/g); M2 showed no detectable additives. Toxicity tests indicated no significant effects on *D. magna*, *T. thermophila*, or buckwheat growth. *L. minor* exhibited a non-significant trend of chlorophyll b reduction (~22–27%). No ingestion of MPs by *T. thermophila* was observed. Overall, MPs from plastic bags contain additives that may leach into aquatic media, but under tested conditions, acute toxicity was absent. Slight effects on aquatic plants suggest potential relevance for chronic exposure. The presented leaching approach simulates high-concentration scenarios under environmentally relevant conditions, providing a basis for future risk assessments of biodegradable plastics.

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2.13.P-We142 A Western-style diet shapes the gut and liver responses to low-dose, fit-for-purpose polystyrene nanoplastics in mice

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The potential hazards of nanoplastics to human health are not well understood, and multidisciplinary studies are crucial to addressing such complex issue. The likely presence of nanoplastics in our diet indicates that ingestion is a major exposure route, with the intestine being the primary organ affected. However, research in this area remains limited and assessing human health risks from oral exposure to nanoplastics is a pressing public health concern. Additionally, existing studies mainly rely on commercial nanoplastic models, whose synthesis processes are poorly described, complicating the assessment of their actual toxicity.

In this context, we used fit-for-purpose gold-labelled polystyrene nanoplastic models, synthesised without chemical additives. We investigated the impact of nanoplastic exposure on the digestive environment in a preclinical in vivo model under two dietary conditions: a control diet and a Western diet, to mimic the conditions experienced by at-risk overweight populations. Based on existing literature, adult male mice were exposed to different doses of nanoplastics (0.1, 1 and 10 mg/kg body weight per day) in drinking water for 90 days.

Low-dose exposure to additive-free polystyrene nanoplastics induced alterations along the gut-liver axis, whereas higher doses had little effect. In the intestine, low doses modulated tight junction gene expression and altered mucus quality through mucin O-glycosylation (i.e. increased mucin sialylation), without inducing measurable changes in mucus quantity. Low-dose exposure also shifted the gut microbiota composition in a diet-dependent manner, with an enrichment in taxa known to feed on sialylated substrates, consistent with mucin O-glycan profiles. Metabolomic changes were only detectable under the control diet, indicating disrupted microbial metabolism.

Overall, low-dose exposure to nanoplastics in their particulate form, in the absence of chemical additives, induces effects on the intestinal barrier, including the gut microbiota, as well as on liver function, particularly with regard to glucose and lipid metabolism. Interestingly, these changes are not observed at higher doses and depend on the nutritional status. This contributes to addressing the critical need to assess nanoplastic-related health risks to healthy but also at-risk populations. Given the prevalence of Western dietary habits, the interplay between diet, nanoplastics and digestive health is a significant public health concern.

2.13.P-We143 A Risk Prioritisation Approach for Intentionally Added MPs for a UK Regulatory Context

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Due to wide use of intentionally added microplastics (IAMPs) in products across broad use categories (e.g., cosmetic products, detergents, paints and sports infill materials) there is a growing need to understand their environmental risks. This work aims to develop a risk prioritisation approach for IAMPs, to generate risk indicators for UK regulatory purposes.

A national scale risk prioritisation approach for IAMPs has been developed using a new Environmental Risks and Impacts of Chemicals “ENRICH” framework (developed by UKCEH). This combines eco-potency and exposure groupings derived from hazard and exposure assessments of IAMPs from different broad use categories. The risk indicators can be used to explore the relative eco-potency and exposure of different broad use categories of IAMPs, as compared to one another, but also as compared to other known chemical exposures in the UK.

There is an absence of specific ecotoxicity data for IAMPs. To establish eco-potency groups in the risk indicator, results from an assessment of two alternative approaches will be presented. Ultimately, a data re-alignment approach was used to estimate provisional effect thresholds (HC50) from species sensitivity distributions (SSDs), specifically for MP particle driven effects. An eco-potency grouping was assigned to particle driven effects of IAMPs as a class, using an existing SSD database, comparing MP HC50 to other chemicals.

For exposure assessment, the UTOPIA model was used to predict environmental concentrations of IAMPs released from different product uses (presented elsewhere). These were compared to concentrations of other chemicals in soils and waters, to assign an exposure grouping for each broad use category of IAMPs. Both eco-potency and exposure groups are combined to create a relative risk matrix, supporting decisions whether IAMPs should be prioritised for regulatory or policy actions over other pollutants in the same environment. Results show that sports infill materials have the highest relative risk indicator in UK freshwaters, in comparison to other IAMPs, such as cosmetics and detergents. This is driven by higher emissions and exposure in freshwaters of this broad use of IAMPs.

2.13.P-We144 Size Matters: Challenges and Recommendations for Reliable Microplastic Size measurement

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Reliable size determination of microplastics is essential for interpreting their environmental behaviour, fate, and ecotoxicological effects. However, there are currently no harmonised recommendations on how microplastic size should be measured or presented. To address this gap, we conducted a systematic literature search that included 400 laboratory studies, examining both the techniques used for microplastic size determination and the ways in which size data are reported and treated. The review revealed a wide variety of analytical approaches, with optical microscopy being the most commonly used technique, followed by laser diffraction and other image-based methods. Despite this diversity, the most significant challenges arise from incomplete or inconsistent reporting. In many studies, key information on particle size or data processing was missing. As a result, it is often difficult to interpret experimental outcomes or compare findings across studies when the actual

size characteristics of the microplastics are not reliably documented. To support improved practice, we provide practical examples illustrating common issues in size determination, especially for polydisperse samples and fibrous microplastics, where measurement and data interpretation are particularly challenging. Based on the identified gaps, we propose recommendations for future laboratory research, including considerations of sample size, detection limits, appropriate size metrics, quality control, and consistent reporting standards. Adopting these recommendations will enhance the transparency, reproducibility, and comparability of microplastic size data, ultimately strengthening exposure assessments and the interpretation of microplastic effects in environmental and ecotoxicological studies.

2.13.P-We145 Tire Rubber Particles Leachate Toxicity in Freshwater Ecosystems: Behavioral, Predator-Cue Responsiveness and Developmental Impairments in *Physa acuta*

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Tire rubber particles (TRPs), generated through tire wear, represent a significant yet understudied source of anthropogenic pollution in freshwater ecosystems. Beyond their physical presence, TRPs release complex mixtures of organic compounds, metals and additives into the water, raising concerns about potential ecotoxicological impacts. Increasing attention has been given to behavioural alterations as sensitive endpoints for environmental risk assessment. This study investigated the sublethal and developmental effects of TRP leachates on the freshwater gastropod *Physa acuta*.

Adult snails were exposed to leachates at 0, 1, 5, 15 and 25% for 24 hours (acute) and 7 days (chronic). After exposure, behavioural endpoints, including speed, distance moved and exploration, were quantified before and after the addition of a crayfish (*Astacus astacus*) extract used as a predator cue. In parallel, egg masses were exposed for two weeks to 0, 5, 10, 20, 25, 50 and 100% leachates to assess embryonic development, hatching success and morphological abnormalities.

Adult snails showed clear behavioural impairment at the highest concentration (25%), with marked reductions in locomotion and slower responses to the predator cue, suggesting neurotoxicity or physiological stress. In contrast, low concentrations (1 and 5%) increased activity, potentially indicating nervous system excitation. Predator cue tests revealed that individuals at 25% exhibited diminished antipredator responses, which could increase predation risk in natural conditions. Embryonic exposure resulted in delayed development, reduced hatching rates and increased malformations—such as shell and radula deformities, at moderate concentrations (5 and 10%). At higher concentrations, no development occurred. The LC50 for hatching was 7.9%, equivalent to 0.79 g/L under the experimental conditions, confirming high embryonic sensitivity to TRP leachates.

Overall, these findings demonstrate that TRP leachates cause both acute and chronic toxicity, affecting essential behavioural traits and early developmental stages. Such impacts may compromise population viability and highlight *P. acuta* as a sensitive bioindicator of tire-derived pollution. This study contributes to the growing evidence of ecological risks posed by TRPs and underscores the need to better assess and mitigate their presence in freshwater environments.

2.13.P-We146 Evaluation of Plastic-Water Partitioning Coefficients of Polycyclic Aromatic Hydrocarbons in Seawater and Marine Plastic Wastes in Japan

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Marine plastic waste acts as a vector for environmental pollution, carrying adsorbed hydrophobic contaminants, such as polycyclic aromatic hydrocarbons (PAHs). These pollutants pose significant risks to aquatic organisms through potential transfer via the digestive process. While listed PAHs by the United States Environmental Protection Agency are frequently monitored, investigations into non-listed PAHs are limited. This study addresses this gap by investigating the concentrations and fate of 26 PAHs in both seawater and marine plastic wastes. The total concentrations of PAHs adsorbed on plastic wastes ranged from 8.43 to 5.06×10³ ng g⁻¹ for polyethylene (PE), 4.47 to 1.67×10³ ng g⁻¹ for polypropylene (PP), 27.4 to 1.55×10³ ng g⁻¹ for polystyrene (PS), and 0.63 to 102 ng g⁻¹ for polyethylene terephthalate (PET) collected from Suruga Bay and Osaka Bay, Japan between April 2023 and March 2025. The adsorption trends observed—PE adsorbing higher concentrations than PP, and PP higher than PET—were consistent with previous studies. In the partially burned, pyroplastic-like PE sample, showed that the total PAH concentrations were 2.1 times higher in the burned part than in the unburned part. In seawater samples, the total PAH concentrations ranged from 1.20 to 14.9 ng L⁻¹. These concentrations were

comparable to those reported in the northwestern part of the Japan Sea, but lower than those reported in highly polluted areas such as Liaodong Bay in China or the Elefsina Gulf in Greece. For PE, apparent plastic-water partitioning coefficients (logK_{pw}) values of low molecular weight PAHs were consistent with those in laboratory tests in the previous studies, whereas those of high molecular weight PAHs were lower than the previous studies. This suggests that high molecular weight PAHs may not be in equilibrium or may have undergone degradation, such as photolysis. Conversely, apparent the logK_{pw} values for PS were notably higher than those reported in the previous studies, potentially due to the relatively large specific surface area of the environmental samples. Our investigation demonstrated that PAH adsorption behavior is influenced not only by the material type but also by the specific surface area of plastic wastes. These insights serve as a foundation for understanding the PAH-induced acceleration of microplastic degradation and contribute to more precise ecotoxicological risk assessments of microplastics, considering adsorbed contaminants.

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2.13.P-We147 Toxicity of Tire Rubber Additives 1,3-Diphenylguanidine and 4-Hydroxydiphenylamine to the Water Flea *Daphnia magna*

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Tire rubber (TR) is a semisynthetic polymer with varying chemical properties. It consists of polymer chains and different additives that are used to modify the properties of rubber mass depending on the intended use. These additives are not chemically bonded to the TR and can leach out over time, potentially causing harm in the surrounding environment. Still, the majority of TR-related research focuses on particles rather than the released additives.

In our study, we wanted to assess the ecotoxicity of 1,3-diphenylguanidine (DPG), a commonly used TR vulcanization agent, and 4-hydroxydiphenylamine (HDP), a hydrolysis product of TR antiozonant 6PPD. Our previous work has highlighted these chemicals as possible effectors of the *Daphnia magna* immobilization caused by tire rubber leachate. *D. magna* is a common freshwater crustacean and a vital part of the aquatic food web. We also wanted to compare the toxicity of DPG and HDP to that of tire rubber leachate.

TR leachate was prepared in 10 g/L concentration over a 20-day period (room temperature, darkness, 160 rpm). After leaching, TR particles were removed via filtration (0.22 µm), and concentrations of DPG and HDP were determined with LC–MS/MS. The toxicity of leachate was tested on *D. magna* neonates (< 24h old) in different dilutions (1, 2.5, 5, 10 and 20%) by monitoring their mobility at 24 h and 48 h according to the OECD guidelines. The test concentrations of DPG and HDP were selected to match the TR leachate dilutions both with and without added ZnSO₄ to account for the possible effects of Zn present in the leachate.

Our previous results have shown that 10 g/L TR leachate can induce acute immobilization of *D. magna* already at 5% concentration. Results of this study support that as the lowest observed effect concentration was shown to be 5% with complete immobilization occurring at 10% and 20% dilutions. Of the individual additives, HDP showed clear immobilization at the highest test concentration, especially with the added ZnSO₄. DPG, in turn, had no effect on *D. magna*. We are currently performing a chronic 21-day reproduction test to assess whether the effects of TR leachate and HDP are limited to acute toxicity or if they also have long-term effects. Overall, our study demonstrates the important role of TR-derived chemicals in ecotoxicology and the hazards they may pose to the environment already at low concentrations.

2.13.P-We148 Transcriptomic Signatures of Phthalates Exposure Reveal Potential Dysregulation of DNA Replication in Free-Ranging Mediterranean Fin Whales

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The fin whale (*Balaenoptera physalus*) is the second-largest whale species and one of the most iconic marine mammal in the Mediterranean Sea, one of the most anthropogenically impacted marine basins. Consequently, this population is chronically exposed to both legacy and emerging contaminants, including plastic-derived endocrine-disrupting chemicals such as phthalates. To investigate whether phthalates produce detectable biological effects in free-ranging whales, we analysed the skin whole transcriptome of 20 individuals (13 females, 7 males) and examined gene expression patterns in relation to the concentrations of 11 phthalate

compounds measured in the corresponding blubber samples. Phthalates were quantified via ultrasound-assisted extraction, d-SPE purification, and GC-MS, while RNA-seq libraries were generated following ribodepletion and sequenced on an Illumina NovaSeq X Plus platform. Processed reads were aligned to the *Balaenoptera acutorostrata* genome, quantified with RSEM, and analysed using DESeq2 and correlation approaches, followed by STRING functional analyses.

Mean total phthalate concentration in blubber was 2401.1 ± 1361.5 ng/g l.b., with DBP and DEPH as most abundant compounds. Correlation analysis between transcriptomic data (15,144 genes) and phthalate concentration revealed 115 significant genes (p -value < 0.05), including 46 upregulated and 69 downregulated. Notably, a functionally cohesive subset of upregulated genes enriched for DNA helicase activity and DNA replication pathways was detected. Key components of the MCM helicase complex, replication protein A, PCNA, and RFC subunits showed positive correlations with phthalate levels, suggesting activation of replication-associated stress responses. These transcriptional signatures are compatible with increased cellular proliferation, DNA damage, and potential genome instability.

Overall, our findings indicate that phthalate exposure in free ranging fin whales is associated with significant transcriptional changes in DNA replication that could compromise genome stability and promote neoplastic transformation. The observed transcriptional alteration rise a warning sign regarding potential health implication, highlighting the need for further multidisciplinary studies on this matter.

Disclaimer/Disclosure:

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2.13.P-We149 Investigation of the Adsorption of Dodine on Virgin and Artificially Aged Microplastics Using Ecotoxicological Methods

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Microplastics (MP) are persistent environmental pollutants mainly formed through the aging and fragmentation of plastics. During the aging process, the specific surface area of plastics increases and their surface properties undergo significant changes. MPs also enter the food chain. Their surfaces may be colonized by pathogenic microorganisms, absorb various pollutants them, and various plastic additives originally used in manufacturing could also be released. Our current knowledge of the toxicology of the additives released from them and the pollutants adsorbed on their surface, as well as their transport processes between trophic levels, remains incomplete.

Our research focused on the fungicide dodine and three MPs: polyester (PES 16-20x3000 μ m fiber), polypropylene (PP 20-22x3000 μ m fiber), polyethylene (350 μ m sphere), and their counterparts artificially aged in an aging chamber.

Dodine was incubated in a 2 m/m% NaCl solution at a concentration of 20 mg/L, either alone or in combination with an untreated (virgin) polymer or its aged counterpart (at a concentration of 10 g/L), in the dark for 168 hours with shaking at 160 rpm, and then the solutions were separated from the MPs using a filter cartridge with a pore diameter of 0.7 μ m. The cytotoxicity of the filtrates were tested using a standard acute 30-minute test (ISO 11348), and a chronic test (at 10 and 15 hours of exposure) based on the bioluminescence inhibition in *Aliivibrio fischeri* test organism, and a BLYR bioreporter based on *Saccharomyces cerevisiae*. Virgin and aged PES fibers were examined in a chronic solid phase *Aliivibrio fischeri* test.

In the acute tests among the tested polymers, PES showed the highest toxicity. PE+dodine displayed toxicity comparable to the fungicide control, while PP+dodine and PES+dodine were less toxic thus showing the higher adsorption capacity. In the chronic tests aged PES filtrate induced stronger bioluminescence inhibition than virgin PES filtrate. In the BLYR system, luminescence inhibition decreased by 41% for PES+dodine solution and 77% for aged PES+dodine compared to the MP-free dodine control. Chronic solid phase *A. fischeri* tests confirmed enhanced toxicity of aged PES+dodine ($EC_{50} = 1.13$ mg/L) compared to virgin PES+dodine ($EC_{50} = 7$ mg/L), demonstrating increased adsorption and possible release of toxic substances after aging. We detected the adsorption of dodine fungicide using indirect and direct ecotoxicological methods.

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2.13.P-We150 Synthetic microfiber-active pharmaceutical ingredients interactions: Toxicity in cells of a model oyster species

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Synthetic fibers constitutes a significant fraction of microplastics polluting the oceans, threatening marine life. Considerable loads in urban wastewater endanger species and ecosystems, but also indicate that they can interact with other harmful contaminants amplifying the risk to marine organisms. Therefore, the present study aims to investigate the toxicity mechanisms and modes of action of polyester microfibers contaminated with active pharmaceutical ingredients (APIs) in hemocytes of the oyster *Crassostrea gigas*. Polyester fabrics were washed in a commercial washing machine then the first washing effluent was filtered and microfibers recovered. Fibers were fortified in vitro with amoxicillin (AMX), caffeine (CAF), carbamazepine (CBZ), citalopram (CIT) and orphenadrine (ORPH) to simulate textile fibers and urban contaminants interactions. Selected experimental groups were: control, polyester fibers and polyester fibers fortified with AMX, CAF, CIT, ORPH or CBZ. Primary hemocyte cultures of *C. gigas* were exposed to concentrations ranging from 15 and 250 MFs/ml over 1 day at 17° or 20°C for cellular metabolic activity analyses through the resazurin assay and at 20°C for lysosomal membrane stability evaluation by the neutral red retention time assay. Results demonstrated that polyester fibers did not affect global metabolic activity in both temperatures, however the interaction with CAF increased cells' metabolic activity at 20°C, at lower concentrations (31 and 62 MFs/ml) while for CBZ and CIT the increase was at higher concentrations (62-250 MFs/ml and 125 MFs/ml respectively), these results might be related to the need of maintenance of cell requirements in response to increased pharmaceutical and microfibers concentration, with microfibers acting as a carrier increasing pharmaceuticals internal dose. The opposite occurred for lysosomal membrane stability, where lower concentrations reduced neutral red retention time for polyester microfibers (15-62 MFs/ml), and polyester fibers fortified with CIT (15 and 31 MFs/ml), ORPH (15 and 62 MFs/ml) and CBZ (15 and 62 MFs/ml). These effects might be due to the fact that lysosomes seems to be target for these contaminants and hormesis phenomena. Overall, microfibers interaction with APIs could enhance cellular toxicity increasing the risk of synthetic microfibers pollution. Therefore, to better understand these results, cell morphology and hemocyte-microfiber interactions analyses are intended to be performed.

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2.13.P-We151 Interactive Effects of Surface-Modified Polystyrene Microplastics and P25 TiO₂ Nanoparticles Under UV-A Irradiation: Experiments with a Marine Crustacean *Artemia salina* **Camil Rex M and Amitava Mukherjee, Vellore Institute of Technology, Vellore, India**

Microplastics (MPs) and titanium dioxide nanoparticles (TiO₂ NPs) are increasingly reported in marine environments due to their extensive use and improper waste management. Although natural sunlight contains a substantial ultraviolet-A (UV-A) component that can influence contaminant behaviour, many laboratory toxicity studies rely on visible light conditions, potentially misrepresenting environmental risks. Hence, this study investigated how UV-A exposure modifies the P25 TiO₂ NPs toxicity both in the presence and absence of surface-modified amine (AMPs) and carboxyl (CMPs) polystyrene MPs towards the marine crustacean *Artemia salina*. MPs and TiO₂ NPs were characterised using field emission scanning electron microscopy, dynamic light scattering, and zeta potential analysis. Multiple biological endpoints were assessed, including mortality, oxidative stress responses, lipid peroxidation, antioxidant activity, acetylcholinesterase activity, titanium uptake, and bioconcentration factor. TiO₂ NPs exhibited significantly higher toxic responses than in the presence of MPs. AMPs combined with TiO₂ NPs induced higher mortality than CMPs with TiO₂ NPs, indicating the importance of surface charges in mediating toxicity. In the presence of MPs, titanium uptake and

bioconcentration factor for titanium were decreased, suggesting lower bioavailability of titanium in the presence of MPs. Mixture modelling indicated antagonistic interactions between TiO₂ NPs and functionalised MPs. All these effects were more pronounced under UV-A than under visible light exposure. These results highlight the need to consider realistic light conditions when assessing the ecological risks of photoactive contaminants in marine environments. Overall, the findings revealed that MPs can modify the toxicity of TiO₂ NPs and that UV-A irradiation plays a critical role in influencing their combined effects.

2.13.P-We152 Effect of Dissolved Organic Matter on the Chronic Toxicity of Ultraviolet-weathered Polylactic Acid Microplastics to *Daphnia magna*

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Although biodegradable plastics have been increasingly used as alternatives to conventional plastics, their ability to produce biodegradable microplastics (BMPs) has been reported, and BMPs are known to have similar or even greater toxicity than conventional microplastics (MPs). Numerous studies have also investigated how MP properties change due to various physicochemical and biological factors, as MPs undergo multiple weathering processes in the natural environment, and their toxicity is consequently affected. In this study, we aimed to examine how ultraviolet (UV) exposure affects the toxicity of polylactic acid (PLA) MPs to *Daphnia magna* in freshwater, especially in the presence of natural organic matter (NOM), given that MPs inevitably coexist with NOM in aquatic environments and their interaction alters MP properties. While several studies have shown that NOM can either mitigate or enhance the toxicity of MPs to *Daphnia magna*, the results vary depending on the experimental conditions, and the underlying mechanisms remain uncertain. In addition, the long-term effects of NOM on MPs toxicity have not been extensively studied. To address this knowledge gap, a chronic toxicity experiment was conducted after a UV weathering process, exposing *D. magna* to pristine and UV-weathered PLA MPs with and without dissolved organic matter (DOM). It was hypothesized that UV-weathered PLA MPs would interact differently with DOM owing to UV-induced changes in MP surface properties, therefore altering their chronic toxicity compared to pristine PLA MPs. This study will provide fundamental information for understanding natural factors that influence BMPs toxicity under environmentally realistic weathering scenarios.

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2.13.P-We153 Sub-Lethal Effects of Antibiotics and Microplastics on *Daphnia magna*: Reproduction and Microbiome Disruption

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Antibiotic pollution in aquatic environments is a global concern due to its potential harmful effects on non-target organisms and its contribution to the emergence and spread of antimicrobial resistance (AMR). Equally alarming is the pervasive presence of plastic pollution, including micro- and nanoplastics (MNPs). MNPs can act not only as physical stressors but also as vectors for chemical pollutants, which might potentially alter the bioavailability and ecotoxicity of those other chemical pollutants, including antibiotics. In particular, the absorption of chemicals to these plastics, particularly as the sizes decreases increasing the surface to volume ratio, may lead to increased exposure and bioavailability of specific adsorbed chemical pollutants to aquatic organisms

In this study, we assessed the individual and combined effects of antibiotics and MNPs on the freshwater crustacean *Daphnia magna* assessing both traditional (*daphnia* reproduction) and novel (*daphnia* microbiome composition) endpoints. We examined the individual effects of three antibiotics commonly detected in European surface waters: sulfamethoxazole (SMX), ciprofloxacin (CIP), and azithromycin (AZI), at both predicted no-effect concentrations (PNECs) and elevated levels (10× PNEC), using a 21-day chronic exposure. Given the widespread occurrence of antibiotics and MPs, we also investigated the influence of polyethylene (PE) microplastics (MPs, 10-45 µm, 7 MPs mL⁻¹) alone and in combination with antibiotics to evaluate potential effects of MP-antibiotic mixtures (SMX = 16 and 160 µg L⁻¹; CIP = 60 and 600 ng L⁻¹; AZI = 0.25 and 2.5 µg L⁻¹; 21-day exposure). By integrating ecological toxicity testing with molecular tools, this work aimed to

uncover subtle sub-lethal effects of low-dose antibiotic exposure and their implications for AMR dynamics in freshwater ecosystems.

2.13.P-We154 Influence of Environmental Weathering on Microplastic Toxicity and Cadmium Vector Potential in Mussels *Mytilus galloprovincialis*: Histological, Histochemical and Biochemical Biomarker Assessment

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Plastic pollution is an increasing threat to marine ecosystems, with microplastics (MPs) frequently co-occurring with other pollutants such as trace metals. Weathering alters MP physicochemical properties, potentially modifying their adsorption capacity and toxicity, thereby influencing their role as pollution vectors. This study evaluates the influence of weathering on polystyrene MP adsorption and toxicity, determining the role of weathered and non-weathered MPs as vectors of metal pollution, using Cd as a model pollutant. MPs were obtained by the cryo-fragmentation of both, non-weathered macroplastics (NW), and macroplastics weathered for 10 months in an outdoor seawater mesocosm (W). Prior to the experiment, a Cd exposure was performed to obtain MPs with adsorbed Cd and to determine the maximum metal load adsorbed onto each MP type. Mussels *Mytilus galloprovincialis* were then exposed for 3 d to six different experimental conditions: NW and W MPs (5·10³ particles/mL), MPs with adsorbed Cd (same concentration), and Cd at concentrations corresponding to each MP adsorption capacities (232 µg/L & 60 µg/L). Histochemical (lysosomal membrane destabilization, as labilization period, LP), biochemical (CAT, GST and AChE) and histological biomarkers were assessed. Both types of MPs were characterized by SEM-EDS and ICP-MS. Results showed an unexpectedly reduced adsorption capacity of Cd in W MPs compared to NW MPs, probably related to the competitive behaviour of seawater salts for surface binding sites. In addition, weathering did not show an enhancement in polymer toxicity, which was attributed to the similarity in both particle type properties. Overall, no significant differences in oxidative stress markers or neurotoxic responses were observed in any of the treatments. However, LP and tissue-level alterations (increased number of hypertrophic mucocytes and haemocytic infiltration), were notable responses to the tested pollutants, particularly under Cd exposure, which caused the highest toxic effects. Nevertheless, Cd toxicity decreased when it was adsorbed to MPs, indicating a neutralizing effect in the presence of the polymer. MP alone treatments caused limited or no significant biological response. This work sheds light on the influence of weathering of MPs and their debated role as pollution vectors, providing an integrated view of MP-mediated metal toxicity. Funded: Basque Government (IT1743-22, IT-1446-22) and MICINN (PID2020-118685RB-I00).

2.13.P-We155 Chasing microplastics: Experimental data for modelling their fate in sediments

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Although microplastics are widely recognized and investigated in water bodies, their story continues by entering sediments and by interacting with complex pore structures and slow-moving flows. How far they travel, how quickly they spread and where they become trapped are questions still lacking robust datasets. This work aims to fill that gap by generating experimental data that will support a predictive model describing microplastic transport, dispersion and retention in sediments.

Three sediment types with distinct grain sizes and two microplastics with contrasting densities—polyethylene (density 0.917 ± 0.052 g/mL) and polylactic acid (density 1.459 ± 0.037 g/mL)—were examined. Prior to transport experiments, sediment porosity, dispersivity, permeability and grain size distribution were measured. Experiments were conducted in a horizontal glass column (5.5 cm diameter; 60 cm sediment bed length) with flow driven by a controlled head difference between inlet and outlet tanks, resulting in flow rates of 0.25–1 mL/s, comparable to riverbed sediment conditions. Microplastics were introduced at the inlet of the column, and each experiment continued for four pore volumes to track their migration through the sediment. After each experiment, the column was sectioned to recover microplastics using water for polyethylene and saturated calcium nitrate solution for polylactic acid. Extracted particles were then stained with Nile red to differentiate microplastics from sediment grains, observed under a fluorescence microscope and enumerated to determine their spatial distribution.

Results indicate distinct transport behaviour between low-density and high-density microplastics and highlight

the strong influence of sediment grain size on retention. These data provide key empirical parameters for developing predictive fate models of microplastics in sediments. Such models, together with standardized experimental protocols, can support efforts to forecast contaminant behaviour in aquatic environments and help prioritize sites for monitoring and remediation.

2.13.P-We156 Nitrogen–microplastic interactions shaped by biofilm development on conventional and biodegradable microplastics

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Microplastics are now recognized as ubiquitous components of aquatic ecosystems, where they interact with organisms and influence water quality, sediment properties, and nutrient dynamics. Although biological and chemical interactions involving microplastics have been widely investigated, their interactions with nutrients remain understudied. Here, we examined how three nitrogen species, namely ammonium nitrogen, nitrite, and nitrate, interact with two microplastic types: conventional polyethylene (PE) and biodegradable polybutylene adipate terephthalate blended with starch (PBAT). To assess the influence of biological conditioning, both pristine and biotically aged particles were tested. Our results show that biofilm presence and viability strongly modulate these interactions. Ammonium nitrogen associated with aged PBAT in substantially lower quantities compared with pristine PBAT, whereas PE showed no notable biofilm-related changes. In contrast, viable biofilm enhanced nitrite association with both polymers, suggesting microbial uptake rather than simple surface adsorption. Nitrate adsorption was highest on pristine PBAT, while PE with non-viable biofilm exhibited the strongest nitrate adsorption, indicating a shift toward physical rather than biological adsorption mechanisms. Overall, these findings demonstrate that microplastics can interact with nitrogen species in ways that depend strongly on polymer type, aging, and biofilm activity. Consequently, the potential role of microplastics in altering nitrogen cycling and associated processes, such as greenhouse gas emissions, should be further investigated.

2.13.P-We157 Biofilm Formation and Gene Expression in Bacteria Exposed to Microplastics and Plastic-Derived Chemicals

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Micro- and nanoplastics (MNPs) are ubiquitous environmental contaminants that provide habitats for microbial colonization and act as vectors for plastic-associated chemicals. Their surfaces support biofilm formation, which can influence microbial ecology and facilitate horizontal gene transfer. Moreover, plastic additives and degradation products may exhibit toxicities distinct from their parent polymers, increasing the complexity of environmental risk assessment.

This study investigates microbial responses to microplastics and plastic-derived compounds by combining microbiological assays with molecular analyses. The model organisms *Escherichia coli* K12 MG1655 and *Pseudomonas aeruginosa* PAO1 were exposed to polyethylene terephthalate (PET) microplastic particles and carbon black (CB) as a non-plastic reference material. Biofilm formation was quantified using the crystal violet assay, and bacterial growth was monitored photometrically. Total RNA was extracted from planktonic and biofilm-associated cells, transcribed into cDNA, and analyzed for biofilm-related genes (e.g. *rpoS*, *csgD*) using polymerase chain reaction (PCR) and agarose gel electrophoresis.

PET exposure did not inhibit bacterial growth and resulted in neutral or slightly reduced biofilm formation, whereas CB occasionally enhanced biofilm accumulation but interfered with optical measurements due to its pigmentation. PCR analyses indicated differences in gene expression between planktonic and biofilm-associated states, with a weaker *rpoS* signal observed in *P. aeruginosa* biofilms. In addition, benzoic acid, a potential degradation product of PET, significantly reduced bacterial growth and biofilm development, demonstrating that leachates can exert biologically active, potentially anti-biofilm effects independent of particle interactions. These findings highlight material-specific microbial responses and underscore the importance of considering both physical and chemical aspects of plastics in environmental hazard assessments. The combined methodological approach provides a basis for improved mechanistic understanding and supports the development of predictive tools for evaluating complex plastic exposure scenarios.

2.13.P-We158 Exploring the Role of Biofilms on the Co-Occurrence of Material and Chemical Pollution in a Changing World

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Macroplastic pollution is a prominent concern in freshwater systems. Plastic polymers are known sources and vectors of chemical pollutants, concentrating them at levels exceeding their surrounding environment. Treated wastewater emits both plastics and chemical pollutants, with their loading forecast to increase as populations grow and extreme weather events periodically overwhelm wastewater treatment infrastructure. Their co-occurrence is a prominent concern for freshwater ecology.

This study investigates interactions between anthropogenic substrates, microbial biofilms, and pharmaceutical pollution in a treated effluent stream. Anthropogenic material tiles (7cm²) made of three plastic polymers (High density polyethylene, Polypropylene, Polystyrene), aluminium, and glass are deployed to examine variations in biofilm composition across materials. The influence of material type and microbial profile on pharmaceutical assimilation will be assessed. Therefore, this study aims to answer the following research question ‘Are biofilms assimilating pharmaceuticals based upon microbial community and does this change with material?’, with the primary hypothesis being ‘Biofilm pharmaceutical assimilation changes with anthropogenic material’.

These findings will provide novel insights into implications of plastic–pharmaceutical interactions, enhancing understanding of their combined impacts under different hydrological scenarios, informing future wastewater management and ecological risk assessments.

2.13.P-We159 Assessing Microplastic Impact on Soil Microbial Communities: Integrating Metabarcoding Data into Regulatory-Relevant Endpoints

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Microplastics (MP) are emerging environmental contaminants that may affect soil ecosystems, yet their impacts on soil microbial communities remain poorly understood. There is growing interest in developing regulatory endpoints that better reflect actual ecological effects and in incorporating molecular data, such as metabarcoding. This study investigates how exposure to MPs may alter soil microbial structure and diversity using 16S metabarcoding data and explores how these shifts can be translated into regulatory-relevant metrics. The effects of three MPs on the structure and function of the natural soil microbial community were assessed using five nominal concentrations of 0.1, 1, 10, 100 and 1000 mg/kg dry weight (dw) soil applied to a reference soil and a control treatment without particles. The polymer type of all MPs was low density polyethylene (LDPE) and the MPs were fragmented. The particle size ranged from (i) 4.6 µm to (ii) ~500 µm and the larger LDPE particle (ii) was treated with UV irradiation to simulate aging (iii). After 56 days of exposure, we analyzed changes in the microbial composition by high-throughput 16S RNA amplicon sequencing, and the effect on nitrogen transformation by measurements of nitrate in soil extracts following the recommendations of the OECD Test Guideline (TG) 216.

No effect of the small fragmented LDPE particle (i) and the larger fragmented LDPE particle (ii) on the Nitrogen Transformation (OECD TG 216) was observed after 56 days exposure. The lowest observed effect concentration was above the highest test concentration of 1000 mg/kg dw soil. However, after aging of the larger particle with UV irradiation, after 56 days an EC₅₀ of 156 mg/kg dw soil was observed, indicating that the aging of the particle lead to an effect on the soil microbial function. Preliminary differential abundance analysis of metabarcoding data revealed shifts in various taxa in response to microplastic particles, although not all changes followed a clear dose-dependent pattern.

This study found minimal effects of LDPE on soil microbial structure and only modest impacts on specific functional groups, consistent with the functional test observations. These preliminary results provide a foundation for incorporating molecular data into ecologically meaningful thresholds for risk assessment, but further research and method validation are needed.

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2.13.P-We160 Bioplastics in Simulated River Systems: Additive Release and Dissolved Organic Matter (DOM) Characterization

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Bioplastics have emerged as promising alternatives to fossil-based plastics products due to their higher biodegradability and renewable origin. However, a key challenge is that bioplastics often require larger amounts of plastic additives to achieve mechanical and functional properties comparable to those of conventional plastics, raising concerns about their potential environmental impact.

Among the different environments where biodegradation have been investigated, riverine systems are particularly relevant due to their dynamic physical and chemical conditions. Ultraviolet (UV) radiation promotes photooxidative degradation, leading to polymer chain scission, while hydrodynamic forces and interactions with sediments enhance mechanical fragmentation. Together with microbial biodegradation and hydrolysis, these processes play a crucial role in the degradation of plastics and the release of associated additives.

In this study, we simulated riverine biodegradation conditions using continuous water recirculation channels to investigate the leaching behaviour of plastic additives from four different materials: a polyethylene (PE) trash bag and three commercial bio-based materials used for single use applications: trash bags, single use glasses and plates. Released additives were analysed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) via suspect screening analysis. Additionally, dissolved organic matter (DOM) was characterized through high resolution mass spectrometry (HRMS) to assess the extent and nature of the biodegradation processes occurring during the experiment. Among all the materials, the bio-based glass showed the highest number of leached additives (162 over the month-long experiment), while the bio-based plate exhibited the lowest (129). The most leached compound families were plasticizers and polymer-production-related compounds. In terms of biodegradation and in every material, lignin-type compounds increased over the course of the experiment due to their lower biodegradability compared to the other compounds studied.

2.13.P-We161 Differences for effects between crustaceans and fish exposed to tire war particles

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Since the impact of 6-PPD quinone, derived from a tire constituent, on coho salmon was clarified in 2021, research on the biological effects of tire particulates has become active. Subsequently, it has been revealed that the biological impacts of tire particulates are induced not only by 6PPD quinone but also by its isomers and related compounds, and even by the parent compound 6PPD. Tires themselves are manufactured from more than one hundred chemicals. Moreover, there are countless types of tires tailored to various applications, and the substances they contain vary widely. Therefore, many researchers suspect that, in addition to 6PPD quinone, other substances capable of inducing biological effects are also present.

In this study, we introduce the effects of tire ware particulates (TWPs) on aquatic organisms using Cryo-Milled Tire Tread (CMTT) obtained from the U.S. Tire Manufacturers Association. We investigated the impacts on the marine amphipod *Ptilohyale barbicornis* and the freshwater amphipod *Hyalella azteca* when exposed to water suspending TWPs, or to water containing components leached from TWPs. As a result, exposure to suspensions of TWPs produced much stronger mortality effects than exposure to the leachate. The amphipods were found to ingest the particulates, and because the mode of toxicity expression differed between the suspension and the leachate, we considered the possibility that substances retained in the particulates and poorly soluble in water were absorbed in the body after the particulates were taken up, thereby exerting strong effects.

Furthermore, we conducted the same exposures with suspensions and leachates on 2-week-old medaka (*Oryzias latipes*), as we did for the amphipods. At least for the suspensions, medaka exhibited far lower toxicity than amphipods, and almost no effects on growth were observed. These results confirmed a large difference in sensitivity to tire-derived substances between amphipods and medaka.

We also consider the impacts of tire particulates after being released onto roads and degraded, and present changes in effects observed when organisms are exposed to tire particulates that have been irradiated with ultraviolet light for a defined period.

2.13.P-We162 From Algae to Daphnia: Diet-Borne Nanoplastic effects on Transgenerational Transfer and Life-History Analysis

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Nanoplastics (NPs) are known to have the potential to be transferred along the food chain through a feeding-predatory pathway. However, it is unclear how this dietary exposure influences their life-history and its transgenerational toxicity. In this study, a simple model of aquatic food chain was developed using microalgae *Chlorella vulgaris* as primary producer and *Daphnia magna* as first consumer. Microalgae were initially pre-exposed to NPs doped with europium (Eu) at 10 mg L⁻¹ for 5 days. *Daphnia magna* were then fed with a daily dose of 0.1 mg C microalgae per individual daphnia for 21 days. The results showed that *Daphnia magna* accumulated low amount of NPs during the chronic test but exhibited significant developmental damage and reproductive toxicity. A logistic growth model estimated both lower carrying capacity for the exposed group compared to the control group ($p < 0.05$) and non-significant trend of lower intrinsic growth rate. *Daphnia magna* fed with contaminated microalgae showed a significant delay in brood production and average number of neonates per parent ($p < 0.05$). Additionally, NPs were detected in the newly born neonates from exposed *Daphnia magna*, implying a transgenerational transfer of the NPs. Synchrotron-based nanoprobe X-Ray Fluorescence (XRF) further revealed NPs localization in the eggs of exposed *Daphnia magna* which may indicate the migration of ingested NPs from the gut environment to the brood chamber. Together, these findings show that trophic transfer of NPs elicits transgenerational transfer of NPs in *Daphnia magna* and significantly affected their life history, underscoring the need to include dietary routes and generational windows in ecological risk assessments.

2.13.P-We163 Effects Of Micro- and Nanoplastic Exposure on Biological Endpoints as a Function of Particle Characteristics and Species Sensitivity

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Micro- and nanoplastic (MNP) pollution has emerged as a pervasive environmental and food safety issue with documented toxicological effects spanning all levels of biological organization and raising significant concerns for human health. Despite the numerous papers that are published - ±3000 publications in 2024 - the full extent of MNP effects on environmental and human health remain unclear due to the fragmented nature of existing studies. The inherent heterogeneity of MNPs, combined with the high diversity of species, model systems and exposure scenario's used in MNP research, complicates extrapolation of results, hampers cross-study comparisons and comprehensive risk assessment.

To provide an detailed overview of reported toxicological outcomes, relevant publications on MNP effects on different levels of biological organization were retrieved from eight databases, using pre-defined Boolean search strings. The publications were screened for eligibility and quality based on pre-determined criteria. Following a risk-of-bias assessment and selection of publications, critical data elements were extracted in a systematic manner. These elements encompassed bibliographic information, experimental model characteristics, target organ information, detailed specifications of the intervention (treatment and control groups), and the quantitative assessment of biological endpoints (outcomes). A quantitative synthesis was performed to summarize experimental findings and establish the interconnectedness of observed effects. The initial step involved determining the frequency distribution of toxicity endpoints (e.g., oxidative stress, inflammatory responses, membrane integrity disruption, and bioenergetic alterations), correlating them with upstream molecular mechanisms and functionally mapping them onto cellular pathways, such as apoptosis, autophagy, or immune system activation. These findings provide insights into MNP toxicity and facilitate identification of the most common molecular and cellular "hotspots" as a function of particle characteristics and species specificity.

The resulting integrated dataset, linking outcomes to defined Key Events or as modulators of Key Event Relationships, will serve as a foundation for future predictive hazard characterization and quantitative risk assessment. The generated dataset will demonstrate its value through incorporation into existing and novel evidence-based Adverse Outcome Pathway (AOP) networks.

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2.13.P-We164 Mitochondrial Dysfunction Induced by 6PPD-Q and Its Metabolites in Salmonid Fish

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Tire wear particles (TWPs) have been recognized as a significant source of microplastics in urban aquatic environments. After being transported by rainfall, TWPs have been demonstrated to cause adverse effects on

aquatic organisms. Recently, 6PPD-Q, an oxidation product of the widely used tire additive 6PPD, was identified as a toxic chemical causing acute mortality in coho salmon exposed to road runoff. Subsequent studies found that significant acute mortality was found only for salmonid species and there were large differences in sensitivity to 6PPD-Q (>100-fold) even among salmonid species. For example, 24-hours LC50 values of 6PPD-Q were 0.095 µg/L for coho salmon and 0.51 µg/L for white-spotted char, whereas significant mortalities were not observed for chum salmon at environmentally relevant levels of 6PPD-Q. Despite the recent intensive research efforts, the mechanism of 6PPD-Q lethality to sensitive salmonid species remains unclear, although a few molecular and cellular effects, such as mitochondrial uncoupling, disruption of blood-brain barrier, and enhanced vascular permeability, have been reported. In addition, it is still unclear whether 6PPD-Q itself or its metabolites is the key driver for the observed lethality.

In this study, to clarify the molecular and cellular toxicity of 6PPD-Q in sensitive salmonid species, we evaluated the effects of 6PPD-Q and its two major metabolites, 6PPD-Q-1-OH and p-hydroxy 6PPD-Q, on RTgill-W1 cells, a rainbow trout gill cell line. Cytotoxicity for 24 hours was first assessed across concentrations ranging from 62.5 to 2000 µg/L. Subsequently, mitochondrial function was examined at three exposure levels (2, 20, and 200 µg/L), for mitochondrial membrane potential (MMP), intracellular ATP, intracellular calcium, and oxygen consumption rate (OCR). All three compounds reduced cell viability, MMP, and ATP levels, while increased intracellular calcium. OCR did not show significant changes across treatments. Among the tested compounds, p-hydroxy 6PPD-Q exhibited the greatest overall toxicity. These results provide new insights into the subcellular impacts of 6PPD-Q and its metabolites, highlighting mitochondrial dysfunction as a key pathway involved in their toxicological effects. In addition to these experiments, we will present results using crude mitochondria isolated from another sensitive salmonid species, white-spotted char.

2.13.P-We165 Impact of Polystyrene and Polyamide Micro and Nano Plastics on Zebrafish Development

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Micro- and nanoplastics (MNPs) are increasingly detected in the environment, and recent studies indicate the presence of MNPs in different organs, including the reproductive tract. Despite this, the potential impact of MNPs on early-life development remains poorly understood. This study aims to address this gap by assessing the uptake, distribution, and toxicity of different MNPs (commercial polystyrene beads (PS) and custom-made nanoprecipitated polyamide particles (PA)) in zebrafish (*Danio rerio*) embryos. As MNPs are often recognized as foreign particles, our hypothesis is that exposure might trigger innate immune responses.

To test this, we used both wild-type AB and macrophage-specific transgenic zebrafish (Tg(mpeg1.1:EGFP)). For uptake and distribution, embryos (<8-cell stage) were microinjected with 0.5 nL of 5 mg/mL or 10 mg/mL solutions containing either 50 nm or 200 nm fluorescent PS particles, or non-fluorescent PA particles (average size distribution 620 nm), directly into the yolk sac. Uptake and distribution were assessed through automated fluorescence microscopy. Developmental toxicity was assessed in both strains, and transcriptomics was performed in the wild-type strain at non-toxic concentrations to assess early molecular responses. Macrophage recruitment was evaluated in the transgenic line.

Preliminary data show clear distribution of all fluorescent PS particles across tissues in zebrafish embryos, whereas no developmental toxicity was observed with either polymer type. Ongoing analyses will determine whether immune-related pathways are activated at the molecular or cellular level.

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2.13.P-We166 How Tire Particles Affect Fish: Integrating Cellular Stress Responses and Zn Accumulation

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Tire particles have received increasing attention as a considerable source of microplastic pollution in aquatic environments. Tire rubber is a complex chemical mixture of elastomers, metals, fillers and additives, many of which are known to be toxic, although Zn is considered as the key toxicant. Due to the process of vulcanization, which cross-links elastomers through polysulfide bonds into a three dimensional network, tire rubber is practically impossible to recycle using conventional means. For this reason, different methods for rubber devulcanization have been proposed.

Although the ecotoxicology of tire particles have become a major subject of microplastic research, studies in aquatic toxicology mostly focus on acute exposure and only recently the role of particle itself was taken into consideration. Likewise, the biological effects of devulcanized rubber compared to conventional rubber in aquatic organisms are unknown.

For this reason, a 3.6 month long-term feeding experiment was conducted in which rainbow trout (*Oncorhynchus mykiss*) were exposed to two types of tire particles. The cellular responses were assessed by evaluating cytogenetic endpoints, including micronucleus formation, as well as DNA fragmentation via the comet assay. Additionally, metallothionein levels and Zn concentrations were measured in liver tissue. Although genotoxicity increased, hepatic Zn concentrations did not change significantly, indicating that the genotoxic effects of tire particle exposure are not directly related to Zn accumulation.

It is estimated that by 2030, waste will increase to 1.2 billion tires annually, yet some argue that this estimate is far too optimistic, suggesting that the actual number could be more than 4 times higher within the same time frame. While the introduction of Euro 7 regulation will limit the emissions from tire wear within EU, toxicological research should go hand in hand with the development of new recycling methods to ensure safe disposal of anthropogenic waste.

2.13.P-We167 Medium-Driven Charge Shifts and Functionalized Nanoplastics Alter Developmental and Molecular Pathways in an Amphibian Model

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Nanoplastics become emerging pollutants of concern, yet their effects on early amphibian development, despite amphibians' ecological importance and vulnerability, are understudied. We investigated the morphological, behavioral, and transcriptomic responses of *Xenopus laevis* larvae (up to NF45) exposed to clean, dialyzed 200 nm polystyrene nanoplastics functionalized with either carboxyl (PS-COOH) or amine (PS-NH₂) surface groups, alone or in combination, across environmentally relevant concentrations (0.1–10 ppm). Dynamic light scattering confirmed negligible nanoplastics aggregation in the exposure medium, while zeta potential measurements revealed an unexpected medium-induced negative surface charge in PS-NH₂, indicating previously unrecognized charge shifts in specific aqueous exposure conditions. Both nanoplastic types caused low mortality but measurable, charge-dependent morphological disruptions, including reduced body length, head height, and eye area, with PS-NH₂ exerting stronger structural effects in general. Larval behavior remained largely unchanged across different treatments. Transcriptomic profiling provided a comprehensive view of the molecular response of *X. laevis* to nanoplastics, revealing widespread transcriptional downregulation affecting both coding genes and long non-coding RNAs. Distinct pathway signatures were also identified. PS-COOH altered oxidative phosphorylation and ribosomal function; PS-NH₂ induced dose-dependent enrichment in peptide metabolism, cytoskeletal organization, and cell adhesion pathways; and the mixture affected extracellular-matrix-associated processes. Together, these results demonstrate that even clean, dialyzed nanoplastics can perturb amphibian development through charge-specific and mixture-specific molecular pathways. The discovery of medium-driven charge inversion in PS-NH₂ and the identification of global transcriptome repression provide new mechanistic insights into how nanoplastic surface chemistry and text medium shape toxicity, with implications for environmental risk assessment in freshwater ecosystems.

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2.13.P-We168 Amniote Embryos Uncover Interactive Early Developmental Responses to Oppositely Charged Nanoplastics

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Micro- and nanoplastics are increasingly recognized as emerging contaminants, but their molecular effects on developing amniotes remain unclear. Using the chicken embryo as an amniote model, we examined how oppositely charged polystyrene nanoplastics, carboxylated (PS-COOH) and aminated (PS-NH₂) of the same particle size (50 nm), and their mixture influence early development. Both particle types, at sublethal concentrations, impaired embryonic progression and produced abnormal phenotypes, demonstrating charge-dependent developmental sensitivity. Exposure to a mixture of PS-COOH and PS-NH₂ reduced the incidence of deformities relative to single-particle treatments but produced a more pronounced developmental delay. RNAseq analysis revealed that the mixture elicited broader gene expression changes than either particle alone, and produced interaction effects consistent with antagonistic molecular responses. Across treatments, extracellular matrix (ECM) remodeling emerged as a shared pathway, whereas pathway analysis indicated that the mixture produced the strongest activation of canonical signaling pathways and upstream regulators in the embryonic brain. Proteomic analyses further supported charge-specific and mixture-specific effects. PS-COOH altered ECM-associated proteins, such as LARGE1 and LOX, whereas PS-NH₂ affected proteins linked to immune and tumor-related functions, such as IL1B, ATP6V0A2, and SEMA3A. The mixture exposure produced the broadest protein changes, including HIC1, MMS19, and PDE1B, which are involved in processes such as DNA repair and neuronal signaling. Together, these results uncovered that charged nanoplastics and their mixture disrupt embryonic development and molecular pathways in an amniote vertebrate. By integrating developmental, transcriptomic, and proteomic data, our findings highlight charge polarity and mixture interactions as key determinants of nanoplastic effects.

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2.13.P-We169 Toxicological Profiling of Polyethylene Terephthalate Micro- and Nanoplastics and Degradation Compounds in Human Liver Cells

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The growing production and persistence of plastics have led to the widespread presence of micro- and nanoplastics (MNPs) in the environment, raising concerns about their effects on human health. These particles, ranging from 1 nm to 5 mm, possess physicochemical properties that influence their behavior in biological systems. Polyethylene terephthalate (PET), widely used in single-use packaging, degrades into smaller particles and compounds such as bis-2-(hydroxyethyl) terephthalate (BHET), mono-2-(hydroxyethyl) terephthalate (MHET), terephthalic acid (TPA) and ethylene glycol (EG). However, the toxicological impact of these degradation products remains poorly defined. Ingestion is a major human exposure route to MNPs, enabling their accumulation in metabolic organs such as the liver. To clarify their hepatotoxic potential, we evaluated the effects of PET MNPs and PET degradation products on human liver cells (HepG2). Cells were exposed to 12.5-400 µg/mL of PET MNPs and respective degradation products for 24, 48 and 72 h and the effects on cell viability, cell cycle dynamics and production of reactive oxygen species (ROS) were assessed. Results showed that PET MNPs reduced cell viability in a concentration-dependent manner. Degradation products showed marked toxicity at concentrations ≥12.5 µg/mL, with BHET and TPA being the most cytotoxic. BHET, MHET

and TPA altered cell cycle dynamics, increasing the S-phase population and reducing the percentage of cells at G0/G1, whereas PET<12 µm did not significantly affect the cell cycle. All degradation products induced ROS production, indicating oxidative stress associated with decreased viability and cell cycle disturbances. These findings show that PET-derived MNPs and, notably, their degradation chemicals exert cytotoxic and oxidative effects in human hepatocytes. Incorporating both particles and associated chemicals into human health risk assessments is therefore essential.

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2.13.P-We170 Combined Effects of Mercury and Polyester Nanoplastics on Human Lung and Liver Cells

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The environmental persistence of plastic waste leads to the formation of microplastics (MPs <5 mm) and nanoplastics (NPLs <1 µm), increasingly detected in drinking water and food products. Due to their small size and high surface reactivity, NPLs can interact with coexisting pollutants, potentially altering their mobility and toxicity. Mercury is of particular concern because of its persistence and potent toxicity, making the evaluation of combined exposure with NPLs essential for realistic human health risk assessment.

This study investigated the toxicological interactions between Polyester NPLs (PENPLs) and mercury in human lung (A549) and liver (HepG2) cells. Our findings show that binary mixtures produce biological effects distinct from those induced by each contaminant alone. Co-exposure modified the surface properties of the plastic particles, indicating physicochemical interactions capable of altering their cellular behaviour. The mixtures also demonstrated a substantial enhancement of mercury toxicity. Co-exposure to 10 mg/L Hg with PENPLs (both concentrations) led to a marked reduction in mitochondrial activity in A549 and HepG2 cells, decreasing from approximately 83% under single exposure to around 42% in mixtures. Additionally, prolonged co-exposure altered the individual toxicity of each substance even at lower concentrations, revealing interactions that modify expected dose-response patterns. The migratory capacity of both cell lines was also affected differently under mixture conditions, reflecting cell-specific sensitivities not observed during isolated exposures. Finally, oxidative stress occurred only in the mixtures, and increased with mercury concentration, suggesting that NPLs enhance the reactivity of mercury at the cellular level.

Overall, these results show that NPL-mercury mixtures possess unique toxic profiles, including particle surface modification, oxidative stress exclusive to combined exposure and amplified mercury-induced cytotoxicity. These findings underline the need to incorporate mixture toxicity into human health risk assessment frameworks.

2.13.P-We171 Micro- and Nano-plastic Exposure and Placental Health Risks: Evidence Integration within the TRAEC Strategy

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Micro- and nanoplastics (MNPs) have been widely detected in the human body, and accumulating evidence indicates their potential reproductive toxicity. However, their placental health risks and the mechanisms underlying size-dependent responses remain insufficiently characterized. To provide a systematic and quantitative evaluation of their potential risks to placental health, this study adopted the Targeted Risk Assessment of Environmental Chemicals (TRAEC) strategy, constructing an integrated scoring system based on four dimensions: evidence reliability, exposure-level weighting, risk intensity, and direction of association, incorporating both published literature and our experimental findings. Initially, we detected plastic particles in human placental villi using Raman spectroscopy and obtained in situ nanoscale images and complementary

chemical information for individual MNPs through atomic force microscopy-infrared spectroscopy and scanning transmission electron microscope. In parallel, our in vitro experiments demonstrated that exposure to high concentrations (100 µg/mL and 200 µg/mL) of 50 nm and 200 nm polystyrene nanoplastics (PS-NPs) induced cell-cycle arrest and apoptosis in human trophoblast HTR-8/SVneo cells, mediating placental reproductive toxicity through mitochondrial dysfunction. Under the same experimental conditions, exposure to 2 µm microplastic particles did not cause significant functional impairment, supporting a particle size-dependent toxicity pattern. By integrating literature-based evidence with our own experimental findings, the final TRAEC scores indicated an overall moderate level of reproductive toxicity risk, with higher risk estimates for nanoplastics than for microplastics. This study demonstrates the practical applicability of the TRAEC strategy in reproductive health risk assessment of MNPs and provides a methodological framework for understanding the health implications of ubiquitous micro- and nanoplastic exposure in the environment.

2.13.P-We172 Beyond the Polymer: Investigating How Plastic-Associated Chemicals Impact Algal Growth and Crustacean Behaviour

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Plastic pollution poses a critical threat to aquatic ecosystems, with 4-12 million tonnes entering waters annually. While bioplastics offer potential alternatives that degrade up to 20 times faster than conventional plastics, critical gaps remain regarding their leachate toxicity. This study evaluates the ecotoxicological impacts of polylactic acid (PLA), polypropylene (PP), and paper (PAP) leachates using a multispecies approach spanning freshwater and marine environments.

Leachates were generated through passive water migration (10g polymer in 200mL water at 40°C for 10 days) for algal studies and ultrasonic methanol extraction for behavioural assessments. Growth inhibition assays with *Raphidocelis subcapitata* revealed distinct material-specific toxicity profiles: PP caused severe inhibition (62% at full concentration), PLA showed minimal toxicity (4.2% inhibition), while PAP unexpectedly stimulated algal growth (-17%), raising eutrophication concerns. Ongoing behavioral studies with hermit crabs (*Pagurus bernhardus*) assess sublethal effects through startle response experiments (cognitive function) and affiliation/avoidance trials. Preliminary results with oleamide, a plastic additive, showed strong crab attraction, suggesting plastic-associated chemicals may create "ecological traps."

These findings demonstrate that "bioplastic" does not guarantee environmental safety and that material-specific responses require individual evaluation beyond biodegradability metrics. This research establishes a holistic assessment framework combining chemical analysis, toxicity assays, and behavioural endpoints to inform evidence-based policy on sustainable plastic alternatives, ensuring material choices genuinely reduce environmental harm across aquatic ecosystems.

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2.13.P-We173 Does the Size of Incubated Biodegradable Mulch Microplastics Alter Lettuce In Vitro Growth?

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The use of biodegradable mulches in agriculture is expanding because its biodegradability offers an advantage over conventional plastics used for mulching, which end up accumulating microplastics in the soil. As they degrade within soil into microplastics, they can release compounds and modify soil properties that may harm plants and soil life. In vitro systems allow studying the effects of the microplastics and of the compounds they release on plants, without the complexity of the soil environment. This work aims to assess how microplastics of different sizes from biodegradable mulches, after an incubation period, affect the in vitro development of lettuce plants.

For this purpose, microplastics were produced from a commercial virgin biodegradable mulch made from PBAT-corn starch and vegetable oils, and classified into 4 size fractions: 5-2; 2-0.5; 0.5-0.2 and <0.2 mm. After

UV-sterilisation, microplastics were incorporated into a plant tissue culture MS medium at 0.1% (w/w) and incubated for 8 weeks in a growing room, with a control set without microplastics. Sterilized lettuce seeds were sown and allowed to grow for 4 weeks. Germination was regularly monitored for the first 10 days, the plantlets growth was visually assessed weekly, and the shoot and root weight, length and the photosynthetic pigments were analyzed at the end of the experiment.

Germination proceeded faster in the microplastics treatments while it was very similar to the control treatment at full germination. The microplastics also resulted in longer roots developing at the end of the 4 weeks of culture, the smaller the microplastics, the strongest the effects, despite there being no changes in the pigments' concentration. Microplastics smaller than 0,2 mm also significantly increased root weight. These observations are likely linked to the releasing of di- and tri- glycerols from the biodegradable mulches, which is to be higher for the smallest size microplastics, with larger contact surface with the medium. These compounds may interact in metabolic pathways and in cell elongation, finally enhancing root development.

This work is the first report on the size-dependent after-effects of incubated biodegradable mulches microplastics on plant growth. The results highlight the potential effects the biodegradable mulches microplastics may have on plants, especially the smallest ones, while not exhibiting toxicity.

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2.13.P-We174 Strategic approach to increase environmental realism in leachate testing

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The growing recognition that plastic-associated chemicals, not just the polymer particles themselves, can harm biota has prompted a surge of leachate studies. Reported methods span a wide spectrum of leaching protocols, from environmentally realistic to highly accelerated conditions. Consequently, leachates produce variable biological effects that depend on polymer type, product shape and unknown mixtures of chemicals released. Hence, specific toxicants often remain unidentified.

This review systematically compiles existing leachate-generation, analysis, and testing protocols. It proposes a flexible yet structured workflow for producing environmentally relevant leachates. The workflow is organized around five key elements:

1. Plastic material, e.g. pristine, consumer-grade, recycled or post-consumer items.
2. Pre-leaching steps, e.g. removal of background contaminants.
3. Leaching conditions, e.g. choice of medium, solid-to-liquid ratio, temperature, UV irradiation, duration and mechanical energy.
4. Chemical analysis, e.g. targeted and non-targeted screening, prioritisation of detected compounds.
5. Toxicity testing, e.g. dosing strategy, exposure metrics and endpoints.

Our analysis shows that leaching conditions are tailored to the research question: mild protocols mimic natural exposure, whereas accelerated, harsher conditions enhance detection of low-level chemicals and mimic extended leaching over time. The presented workflow assists researchers to select the most appropriate protocol while maintaining comparability across studies, a step toward harmonised leachate testing.

Nevertheless, several challenges remain. First, the diversity of potential leachates demands comprehensive analytical strategies and prioritisation schemes, yet hazard data are missing for many plastic-associated chemicals. Second, linking toxicity outcomes to specific compounds is complicated by the formation of unknown transformation products during leaching. Third, for end-of-life and recycled plastics, distinguishing intentionally added additives from contaminants adsorbed during the product's life cycle is difficult.

Addressing these challenges requires coordinated efforts in advanced chemical characterisation, toxicological modelling and the development of databases that integrate chemical composition with hazard information. The systematic approach aims to guide future studies toward a clearer understanding of the ecological risks posed by plastic-associated chemicals.

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2.13.P-We175 Microbial, Crustacean and Algal Responses to Leachates of Consumer Plastics: Assessing Toxicity and Biodegradation Potential

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Leaching chemicals from environmental plastic litter is a well-established concern, with the potential to cause negative impacts across all levels of biological organization. The composition and magnitude of chemical release vary widely with polymer type and product-use category, underscoring the need for comparative assessments across plastic types and sources, including new biobased plastics. Here, two environmental bacterial isolates, *Paraburkholderia dioscureae* and *Pseudomonas putida*, a copepod species (*Acartia tonsa*) and a microalgae species (*Rhodomonas marina*) were exposed to leachates derived from five widely used consumer plastic types: polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polylactic acid (PLA), and a biobased starch blend. With this experiment, we analyze whether exposure to these leachates is toxic to the aforementioned species by measuring growth and survival rate, among other endpoints.

The leachates were produced by submerging the plastics in ultrapure water and were subsequently exposed to 20°C, UV light and constant shaking for 38 days to simulate environmentally relevant degradation. Additionally, each leachate was diluted to 50 and 25 % to account for effect differences depending on leachate concentration. Bacterial growth was measured over the course of 72h by optical density (OD). Additionally, bacteria were plated to count the number of Colony Forming Units (CFUs) and control potential contamination by other microorganisms. *A. tonsa* and *R. marina* will be exposed to these but also to ten more additional materials, composed of a mixed of commonly used items and marine relevant materials, such as fishing nets and car tires.

Across all plastic leachates, the growth of *P. dioscureae* was significantly reduced when compared to the negative control after 72 hours (t-test, p-values < 0.05, figure 2), indicating an overall inhibitory effect. For most plastic leachates, growth is already reduced after 24 hours, only the PLA leachate, showed a delayed inhibitory effect with growth being suppressed after the 24 hours. Preliminary findings on bacterial growth reveal differences in leachate effects between product types. These results will demonstrate whether plastic leachates cause ecotoxicological effects. These findings will underscore the complexity of plastic-associated chemical risks and the value of integrating diverse biological models in environmental impact assessments and nature-based solutions.

Disclaimer/Disclosure:

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2.13.P-We176 Microplastics in Regurgitated Pellets of Predatory Birds in Southern England: Have We Been Underestimating Exposure?

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In recent years, microplastics have emerged as pervasive environmental contaminants, raising concerns about exposure levels of wildlife. Predatory birds occupy high trophic positions, and hence are susceptible to the biomagnification of pollutants. There is a rising body of evidence pointing towards birds of prey ingesting microplastics; however, the drivers of exposure are poorly understood. This study is the first to quantify microplastic ingestion by UK raptors. We quantify microplastic ingestion by two raptor species in southern England, barn owls (*Tyto alba*) and kestrels (*Falco tinnunculus*), which share overlapping habitats and diets.

Sampled nests were separated into eight pairs with one nest for each species in the pair (n=16). These paired nests were >10km apart from other pairs, to avoid pseudo replication. Three regurgitated pellets were collected from each nest, totalling 48 pellets. Each pellet was digested with 10% KOH, followed by a Fenton's reaction

and density separation with ZnCl₂. Microplastic presence, size, and polymer composition were analysed using Fourier-transform infrared (FT-IR) spectroscopy.

Out of a potential 21 polymers, 14 were detected with dominant polymers being polypropylene, polyurethane, ethylene vinyl acetate, ethylene vinyl alcohol and polyethylene terephthalate. Microplastic presence was detected in 83% of barn owl and 92% of kestrel pellets. Kestrel pellets contained significantly higher numbers of microplastic particles per gram (20.8 particles/g) compared to barn owl pellets (0.78 particles/g). For both species the number of particles detected increased as particle size decreased, with the highest number of particles found between 25-50 µm.

Our findings demonstrate interspecific differences in microplastic ingestion between barn owls and kestrels. The significant differences in microplastic ingestion between two species that have such closely overlapping foraging ecologies raises important ecological questions, such as whether minute differences in prey ingested can cause significant differences in exposure. Such investigations may allow us to assess which species are at higher risk of exposure based on prey choice. These findings also evidence the value of analytical techniques such as FT-IR spectroscopy, revealing higher ingestion rates than previously recorded using visual identification and demonstrating the prevalence of smaller microplastic particles missed when relying solely on visual identification.

Disclaimer/Disclosure:

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Track 3: Environmental Chemistry and Exposure Assessment: Analysis, Monitoring, Fate and Modelling

3.01 Advances in Bioaccumulation Science and Assessment

3.01.T-01 Development and Evaluation of Data, Models and Tools to Support Bioaccumulation Assessment

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Bioaccumulation (B) assessment is challenging because of measurement data gaps and all B assessment metrics and methods have merits and limitations; uncertainty is inherent. B screening criteria use the octanol-water [KOW] and octanol-air [KOA] partition ratios as surrogates for partitioning to biological phases. Octanol is a reasonable biopartitioning surrogate for many hydrophobic neutral organic chemicals; however, its application for ionisable organic chemicals (IOCs), such as perfluorinated alkyl acids (PFAAs), is limited. Requirements to reduce or eliminate animal testing have prioritized the development of new approach methods (NAMs) employing in vitro and in silico methods, which require evaluation and data generation. In vitro methods include Organization for Economic Coordination and Development (OECD) fish S9 and hepatocyte assays for estimating biotransformation rates (TG 319A/B), which subsequently require in vitro-in vivo extrapolation (IVIVE) and mass balance one-compartment physiologically-based biokinetic (1Co-PBK) models to calculate B metrics. In silico methods also include Quantitative Structure Activity Relationships (QSARs) for B metrics and toxicokinetic parameters, e.g., whole body biotransformation [HLB] half-lives. This presentation provides an overview of recently developed and evaluated databases, methods and tools to support B assessment decision-making including (i) a weight of evidence (WoE) approach to aid B assessment decision-making following OECD (WoE) guidelines and its implementation in the Bioaccumulation Assessment Tool (BAT), (ii) the Bioaccumulation Evaluation Tool (BET) to calculate B metrics in representative aquatic and air-breathing organisms under laboratory and field conditions using in vivo, in vitro and in silico data, (iii) an OECD B Integrated Approach to Testing and Assessment (IATA), (iv) new models for predicting KOW and KOA, (v) new biopartitioning databases and new polyparameter linear free energy relationships (PPLFERs) for predicting

chemical partitioning to storage lipids, membrane lipids, structural proteins and serum albumin, and (vi) updated IVIVE models for using S9 and hepatocyte data to parameterize 1Co-PBK models. The presented case studies outline possible future IATAs for biopartitioning and biotransformation rates which are Key Areas of Regulatory Challenge identified in the scientific community and by the European Chemicals Agency (ECHA).

3.01.T-02 Application of EAS-E Suite to Inform In Silico-Based Assessments of Bioaccumulation in Air Breathing Organisms: Case Study with Hydrocarbons in Laboratory Rat

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Bioaccumulation (B) assessments have traditionally focused on aquatic organisms, but regulatory agencies are increasingly considering air-breathing species. The European Chemicals Agency (ECHA) outlines a framework for assessing B in air-breathers (mammals): initial screening by physicochemical properties (i.e., octanol-water [KOW] and octanol-air [KOA] partition ratios); refined screening using quantitative structure-activity relationships (QSAR) or in vitro toxicokinetic (TK) data (e.g., biotransformation [HLB] or elimination [HLT] half-lives); and definitive in vivo testing. Because empirical data and standardized in vivo methods for assessing B in air-breathers are limited (and animal testing conflicts with 3Rs principles), assessments are expected to rely on in silico and in vitro new approach methods (NAMs). We applied the Exposure And Safety Estimation (EAS-E) Suite to evaluate the B potential of 1981 unique hydrocarbons (HC) in a representative air-breathing organism (laboratory rat). Key data (i.e., KOW, KOA, HLB, HLT) were compiled from available measurements or predicted using multiple QSAR models. Central-tendency estimates (e.g., mean, median, SD) for each parameter were incorporated into a one-compartment physiologically based TK model to estimate biomagnification factors (BMF) with propagated uncertainty. When biotransformation was excluded, 60% of HCs exceeded the regulatory BMF threshold of 1, while the rest had reduced dietary uptake efficiency due to their high hydrophobicity ($\log KOW > 12$). As most hydrocarbons are expected to undergo biotransformation, incorporating HLB predictions substantially reduced median BMFs for all HCs to below 1. When 95% confidence intervals were included, 88% of HCs had upper-bound BMFs below 1, suggesting they may be deprioritized for further B assessment. 90% of the variation in BMF estimates was driven by HLB, underscoring the critical influence of biotransformation in determining B potential. For HCs with upper-bound BMFs above 1, the uncertainty may reflect compounds falling outside the applicability domain of HLB QSARs, particularly aliphatic HCs, and underscores the need for additional data to improve QSAR-based assessments of these substances. This work illustrates how in silico NAMs can be effectively integrated into a tiered framework for assessing B in air-breathing organisms.

3.01.T-03 fishFingers: Bioconcentration factor (BCF) and fish species bioaccumulation distribution prediction for chemicals detected by HRMS using recommender system machine learning

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Exposure to novel entities in the environment now exceeds our capacity to evaluate the risk of each chemical under traditional frameworks. Detecting, prioritising and identifying thousands of anthropogenically-derived chemicals already present in the environment is a key challenge to understand this planetary boundary. The application of machine learning to chemical risk management has already demonstrated quick and accurate ecotoxicological characteristics of chemicals with low confidence structural annotation inferred from high-resolution mass spectrometry (HRMS). The rate at which a chemical is absorbed into an organism compared to the rate it is excreted, known as the bioconcentration factor (BCF), is also an important factor for evaluating chemical risk. Since toxicity and bioaccumulation are often used to regulate chemicals, accurate and precise in silico techniques can greatly enhance our capacity for chemical risk assessment. Unfortunately, all known methods of reliable BCF prediction require a confident chemical structure. The prediction of BCF from HRMS features without a priori structural annotation will be demonstrated, using high-quality tandem mass spectrometry (MS2) to elucidate key structural fingerprints. This offline prioritisation method for non-targeted screening can quickly rank features in order of decreasing risk of bioaccumulation. A dataset containing measured BCF values for more than 1000 chemicals was obtained and curated for training and testing. For each chemical, a matrix of 2691 structural fingerprints were calculated using the redk package, incorporating MACCS, PubChem, Klekota-Roth, and a number of custom SMARTS. A recommender system approach was applied to model BCF with inputs from structural fingerprints from over 100 species of fish. The performance of linear regression, decision tree and dropout tree algorithms were compared, and the best performing model was optimised and tested to achieve the highest balanced accuracy, calculated as $(TPR+TNR)/2$. Chemicals with high-quality MS2 in the MassBank spectral library were then used to simulate HRMS results and validate the

model using SIRIUS CSI:FingerID v5.8 to generate the structural fingerprints using the averaged peak lists. The performance of the model was compared with methods described in the literature, including hydrophobicity-based models (log Kow) and other popular QSAR models that use known structural annotations.

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3.01.T-04 Gammarus fossarum as European freshwater amphipod species for bioconcentration application

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Bioconcentration factor (BCF) is the ultimate decisive bioaccumulation criterion as part of the regulatory chemical safety assessment of pollutants in aquatic ecosystem (REACH regulation). The Hyalella azteca Bioconcentration Test (HYBIT) - that aims to determine BCF in the North-American amphipod H. azteca for regulatory purposes - provides a non-vertebrate alternative to fish bioconcentration studies. Here, we aim to highlight the interest of the European amphipod Gammarus fossarum as model in HYBIT test guideline (TG) 321 that has been recently adopted at OECD. To do so, we initiated in 2025 an interlaboratory assay (Ring-test) with three European countries to determine the kinetics of bioaccumulation and depuration of terbutryn (herbicide of the triazine family) and prochloraz (fungicide of the imidazole family), two molecules that were previously selected for H. azteca validation test. During experiments, amphipods are exposed to chemicals dissolved in water for 4 days (uptake phase; nominal concentration: 50 µg.L⁻¹), then transferred to controlled conditions for 4 days (depuration phase). Pools of individuals and water samples are collected and analysed at different intervals. Results from three laboratories showed that the mortality rate remained below 20% regardless of the treatment (mean ± SE: 9.2 ± 1.6%, 11.1 ± 3.7% and 12.8 ± 2.1%, respectively for control, terbutryn and prochloraz), which meets the corresponding validity criterion of the HYBIT protocol. Test solutions and gammarid tissue analyses from one laboratory allowed us to calculate BCFs values (BCF = 0.8 and 20 respectively for terbutryn and prochloraz) which indicated that prochloraz bioconcentrated more than terbutryn in G. fossarum, as previously shown for H. azteca. First conclusions of the Ring-test will be presented during SETAC 2026 conference.

Key-words: ecotoxicology; laboratory exposure; bioconcentration factor; HYBIT; OECD; gammarids

3.01.T-05 Trophic Transfer and Interactions of TiO₂ Nanoparticles in a Simplified Aquatic Trophic Chain

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Titanium dioxide nanoparticles (TiO₂ NPs) are widely used in several domains and can enter aquatic systems, where natural organic matter modifies their stability, aggregation and biological uptake. While waterborne toxicity of TiO₂ NPs is well studied, less is known about dietary transfer across trophic levels. Therefore, in this study, we aimed to examine the trophic transfer of Humic Acids-coated TiO₂ NPs (21 nm) along a three-level chain comprising the microalgae "Tetraselmis suecica", the brine shrimp "Artemia" sp. and the zebrafish (Danio rerio). In addition to bioaccumulation/quantification of total titanium and NPs in tissues of three trophic levels (studied by ICP-MS/sp-ICP-MS), biochemical markers (LPO, GST, CAT), energy content, and C:N ratio were assessed.

Microalgae exposed to TiO₂ NPs accumulated notable Ti concentrations (~14 µg g⁻¹), though <2% was detected as discrete particles, reflecting strong aggregation in saline media and surface attachment to cells. Artemia accumulated low and variable Ti (<1 µg g⁻¹), suggesting rapid clearance, yet zebrafish still showed clear

evidence of dietary uptake. Ti levels in the zebrafish gastrointestinal tract were about twice those of controls, confirming trophic transfer to the highest consumer despite the aggregated state of the particles. Single-particle ICP-MS signals were often below detection, indicating that Ti reached fish mainly as non-particulate or aggregated forms rather than as individualized NPs. Lower-trophic-level nutritional markers showed minor changes (e.g., non-significant C:N increase in *Artemia*) and require additional attention. In zebrafish, no statistically significant alterations were detected in muscle energy reserves or liver oxidative stress markers ($p > 0.05$); however, subtle tendencies (e.g.: marginally higher lipid peroxidation and reduced catalase activity) suggest antioxidant responses that could intensify under longer exposures or higher dietary loads. Ongoing analyses are assessing cellular alterations in zebrafish gonads (histology) as well as lipid and FAME profile changes resulting from dietary exposure to TiO_2 NPs.

Overall, TiO_2 NPs rapidly aggregated but still adhered to microalgae and were transferred through the diet up to zebrafish. Although biological impacts were not fully clear, the demonstrated bioaccumulation at the top trophic level highlights the ecological relevance of dietary exposure pathways and the need for longer-term, tissue-specific assessments.

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3.01.P Advances in Bioaccumulation Science and Assessment

3.01.P-Mo140 Proposal for an Integrated Assessment Strategy for Aquatic and Terrestrial Bioaccumulation

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An assessment strategy for evaluating bioaccumulation in aquatic and terrestrial organisms is introduced. Building on existing regulatory guidance, the strategy combines *in silico*, *in vitro*, and invertebrate approaches with existing *in vivo* knowledge to minimise vertebrate testing while ensuring the quality of regulatory decisions. It covers neutral lipophilic, ionisable, superhydrophobic, and amphiphilic substances, providing both a stepwise pathway for new chemicals and a weight-of-evidence approach for existing substances. The strategy consists of a tiered aquatic system and a parallel system for air-breathing organisms. In the aquatic scheme, tier 1 relies on hydrophobicity indicators (log K_{ow} or log D for ionisable substances). Tier 2 uses QSAR-based BCF estimates, with consolidation of multiple models to account for variability. Tier 3 introduces mechanistic non-vertebrate and *in vitro* tests, and tier 4 requires fish testing (OECD 305) only in cases where the available information from earlier tiers remains inconclusive. The scheme also addresses non-lipid-driven accumulation relevant to PFAS, ionisable substances, and surfactants. The terrestrial scheme incorporates log K_{oa} and metabolic capacity metrics to assess accumulation potential in mammals and birds. The integrated strategy was evaluated using 26 case study substances by comparing results from QSARs, OECD 319A/B, OECD 321, and OECD 305 tests. For moderately to highly hydrophobic chemicals, discrepancies between *in vitro*, invertebrate, and *in vivo* results highlighted the need for expert interpretation, and for superhydrophobic substances, methodological limitations reduced the applicability of current NAMs. Ionisable substances, PFAS, and surfactants also required specific considerations. For air-breathing organisms, rat hepatocyte and S9 assays showed promise, and a preliminary tier 2 screening threshold was identified, though further refinement is needed. In summary, the integrated testing and assessment strategy proved effective for identifying substances of bioaccumulation concern and for guiding efficient testing sequences that can be used across EU substance regulations. It confirmed the complementary roles of *in silico*, *in vitro*, and invertebrate methods and highlighted the importance of expert judgement for complex cases. NAMs can substantially reduce vertebrate testing while maintaining regulatory protection levels.

3.01.P-Mo141 Refining the Bioconcentration Testing Trigger: A Weight of Evidence Approach

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Environmental risk assessments for human medicines require fish bioconcentration testing (OECD 305) when $\log K_{ow} \geq 3$. If bioconcentration factor (BCF) >100 L/kg, the risk for secondary poisoning is assessed. Industry data show ~60% of triggered OECD 305 studies for human medicines yield $BCF_{KgL} <100$ L/kg, the regulatory threshold for secondary poisoning assessment. K_{ow} poorly predicts bioconcentration of human medicines due to factors beyond hydrophobicity (e.g., metabolism, active transport, molecular properties). Since most human medicines trigger unnecessary testing, significant opportunity exists to apply 3R principles. Using a small dataset of pharmaceuticals with measured BCF, we evaluated potential screening tools that could be used in a weight-of-evidence (WoE) approach to determine when to conduct an OECD 305.

One tool is tissue analysis of fish from a required early-life stage study (OECD 210). Tissue samples collected from 14-day post-hatch fathead minnows at the conclusion of the OECD 210 range-finding study, utilizing fish typically discarded after growth measurements. Another tool is a toxicokinetics study, that facilitates read-across between fish and preclinical species, where plasma and tissue concentrations are measured over a 14-day exposure and a 5-day clearance. For 5 pharmaceuticals evaluated, the toxicokinetic and/or OECD 210 body burden measurements accurately predict BCF. For molecules with OECD 305 $BCF_{KgL} <100$ L/kg ($n=4$), body burden show BCF below the regulatory threshold (range: 0.2-24 L/kg). For compounds >100 L/kg ($n=1$), the approach correctly identified a secondary poisoning potential (range: 188 to 241 L/kg). When used in combination, no false negatives occurred.

We also evaluate the use of intrinsic clearance by fish hepatocytes (OECD 319A) as a screening tool. When $\log K_{ow}$, hepatocyte clearance and body burden data indicate BCF >100 L/kg, an OECD 305 is conducted to evaluate secondary poisoning risks.

This proof-of-concept WoE approach demonstrates that pharmaceutical bioconcentration can be reliably predicted using tissue from fish already sacrificed for required testing. The approach eliminates redundant vertebrate use, saves ~10 mCi radiolabel, and maintains regulatory confidence through validated methods. With regulatory openness to 3R solutions backed by data, implementation could reduce unnecessary vertebrate use without increasing the risk of secondary poisoning.

3.01.P-Mo142 Small aquatic invertebrates in bioaccumulation assessment

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Current bioaccumulation assessments primarily depend on fish data, which are limited by kinetic constraints, as steady-state bioaccumulation factors are often calculated without confirming that steady-state conditions have been achieved. These experimental shortcomings compromise the reliability of QSAR models developed for *in silico* bioaccumulation predictions. Testing is particularly challenging for highly hydrophobic compounds due to difficulties maintaining stable exposure and long time needed to approach steady-state. The Environmental Chemicals Agency has recently identified improved bioaccumulation testing methods as one of the key regulatory challenges. However, ethical constraints and low throughput make fish tests ill-suited to addressing this challenge.

Smaller organisms achieve steady-state more rapidly than fish. *Daphnia magna*, well-established in laboratory and regulatory practice, exhibits depuration rates approximately 100-fold faster than fish, enabling substantially shorter test durations. We recently developed a testing method maintaining constant exposure at very low concentrations (ng/L range) while simultaneously assessing chronic toxicity and bioaccumulation.

The data requirements for advancing this approach toward standardized test procedures were systematically evaluated and the key advantages include: rapid uptake/depuration kinetics, small organism and housing container size, potential integration with chronic toxicity assessments, lower ethical constraints, and potential for dense sampling to obtain detailed kinetic profiles. Identified limitations include: limited tissue mass for chemical analysis, insufficient physiological data for mechanistic modeling, and periodically occurring moulting and offspring release.

To address critical data gaps, we characterized key *D. magna* physiological traits including: wet/dry weight dynamics during growth (yielding growth rate parameters), body composition, and feeding rates. These data enable assessment of different uptake and depuration routes using physiologically-based toxicokinetic (PBTK) models.

Our physiological characterization provides essential parameters for PBTK modeling, advancing the scientific foundation for standardized *Daphnia*-based bioaccumulation assessment methods. *Daphnia magna* represents a promising alternative platform for bioaccumulation testing, offering significant practical and ethical advantages over traditional fish tests.

3.01.P-Mo143 Implications of Variable Octanol-Water Partition Coefficient (KOW) Values in Bioaccumulation Assessment

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The octanol–water partition coefficient (log KOW) is both an intrinsic substance property and an input parameter for modelling, e.g. for quantitative structure-activity relationships (QSARs) and in vitro-in vivo extrapolations (IVIVE). These methods are increasingly used to reduce animal testing (following the 3R principles). Errors in log KOW values can therefore be critical if propagated in bioaccumulation assessments. When evaluating substances against regulatory thresholds, such as a log KOW value of 4.5, high-quality data is often not required. Where two log KOW estimates for a substance are clearly below this value, e.g. 1 and 3, or above it, e.g. 6 and 8, no further refinement is needed for a robust conclusion. Less variability is essential if the log KOW is within one log unit below or above the screening value of log KOW = 4.5. Then, consolidation of the log KOW value by consensus modelling can reduce uncertainties and is superior to the traditional preference for a single measured log KOW.

QSARs aim to describe and predict the bioaccumulation of substances from their chemical structures.

Considering bioconcentration as a distribution between aqueous and organic phases has produced many direct relationships with log KOW as a worst-case approach. There, log KOW error ranges of ± 0.3 or ± 0.5 may make the difference in the predictions of log BCF of substances being either non-bioaccumulative (BCF < 2000) or (very) bioaccumulative (BCF > 2000/5000).

One of the new approach methods (NAMs) is based on in vitro substrate depletion assays (OECD 319 A/B). It determines biotransformation rates in fish liver S9 fractions or hepatocytes. These are used to predict BCF values with IVIVE. Their input variables include the experimental specifications and measured biotransformation rates as well as log KOW values, which are intended to represent the distribution behaviour. Using case study chemicals, we will illustrate the consequences of variability in log KOW values for IVIVE-based BCF values. These will be compared with the predictivity of an empirical regression model based on the in vitro intrinsic clearance and log KOW.

Recommendations will be presented on appropriate methods to consolidate log KOW (fit-for-purpose).

Integrating multiple lines of evidence from refined physicochemical parameters, QSARs and NAMs can jointly provide reliable information for bioaccumulation assessments.

3.01.P-Mo144 Predictivity of Physicochemical Properties and Quantitative Structure-Activity Relationships (QSARs) in Tiered Bioaccumulation Assessments

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The evaluation of bioaccumulation potential is one of the fundamental elements of environmental risk assessment for chemical regulation. Integrated Approaches to Testing and Assessment (IATA) have multiple tiers that combine in silico, in vitro, and invertebrate testing with established in vivo data, and aim at reducing the need for animal testing and improve regulatory efficiency.

At Tier 1, hydrophobicity is generally assessed using the octanol–water partition coefficient (log KOW) as the primary indicator for aquatic bioaccumulation potential. Comparative analyses revealed substantial variability in log KOW values determined with different experimental and computational methods. Iterative consensus modelling was found to reduce uncertainty and to provide more stable input values for bioaccumulation assessment. Furthermore, log KOW in combination with the octanol–air partition coefficient (log KOA) and biotransformation half-life, relate to the terrestrial accumulation potential. For low log KOA, the limited variability (within one order of magnitude) supports a reduced approach to assess substances against the threshold of 5 for unlikely bioaccumulation in air-breathing organisms.

At Tier 2, bioconcentration factors (BCFs) are usually estimated using QSAR models available, e.g., in EpiSuite, VEGA, OCHEM, etc. Variability among model outputs is notable, with differences of more than one log unit observed for some substances. Consolidation of QSAR results across datasets allowed estimation of uncertainty ranges and improved prediction reliability. However, further validation of relevant QSARs for regulatory purposes is necessary.

Using case study chemicals, we will illustrate the consequences of variability in input data on physicochemical properties and the benefits of QSAR-based classifications of substances as either not-bioaccumulative (nonB) or (very) bioaccumulative (B/vB). The focus is on REACH chemicals, including examples of superhydrophobics and a volatile substance that is likely to biomagnify in terrestrial food webs.

3.01.P-Mo145 Optimization of the Slow-Stirring Method for Measuring 1-Octanol/Water Partition Coefficients of Surfactants and Application to Bioaccumulation Assessment

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The 1-octanol/water partition coefficient (log Pow) reflects the hydrophobicity of organic chemicals and is used to predict their environmental behavior and toxicity. In particular, log Pow is a key parameter for estimating the bioconcentration factor (BCF), a critical indicator of bioaccumulation potential.

However, a reliable method for log Pow measurement has yet to be established for surfactants, due to their unique properties such as micelle formation, emulsification, and adsorption. Thus, log Pow is considered unreliable for estimating BCFs of surfactants.

Due to their amphiphilic nature, surfactants interact with biological membranes, affecting their distribution and accumulation. Therefore, measuring phospholipid membrane-water partition coefficients (log Km_w) may help estimate surfactant bioaccumulation, though derivation can be complicated by strong sorption, structural diversity, and membrane interactions.

In this study, we optimized the measurement conditions of the slow-stirring method for determining log Pow of surfactants. Previous studies have reported that this method can be applied to surfactants when the aqueous concentration during equilibration remains below the critical micelle concentration.

Furthermore, to evaluate the utility of log Pow measured by the slow-stirring method, we conducted regression analyses examining relationships between log Pow by the method and experimentally determined BCFs for anionic and cationic surfactants.

As a result, optimizing the initial concentration and stirring conditions enabled stable and reproducible measurement of log Pow by the slow-stirring method. For anionic surfactants, BCFs could be estimated using a simple linear regression equation based on log Pow measured in this way. In contrast, accurate BCF estimation for cationic surfactants required molecular weight as a descriptor in addition to log Pow obtained by the slow-stirring method.

Moreover, a good correlation was observed between log Pow measured by this method and log Km_w, suggesting that log Pow determined by this approach may be a useful indicator for quantitatively assessing the bioaccumulation potential of surfactants.

In conclusion, the optimized slow-stirring method established in this study enables appropriate measurement of log Pow for surfactants and offers an effective approach for assessing bioaccumulation potential. This method is expected to contribute to chemical regulation and environmental risk assessment frameworks.

3.01.P-Mo146 The Influence of Biological Traits on Bioconcentration of Organic Chemicals in Freshwater Invertebrates

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Organic chemicals released into aquatic environments, including pesticides, pharmaceuticals and personal care products, may pose ecological and human health risks. Uptake of organic chemicals can vary widely across aquatic species, yet the biological traits driving these differences remain poorly understood. Traits such as size, lipid content, body structure and respiration likely play a key role in chemical accumulation, but they are rarely incorporated into current risk assessment frameworks. Improving the understanding of how traits influence bioconcentration would strengthen cross-species extrapolation and enhance the ecological relevance of chemical hazard evaluations.

This work aims to develop a trait-based framework for predicting bioconcentration across aquatic invertebrates. Such an approach has the potential to improve ecological relevance, enhance cross-species extrapolation, and support reduced reliance on vertebrate testing in chemical hazard assessment.

This study investigates how biological traits affect toxicokinetics in freshwater invertebrates, using carbamazepine as a model compound. A minimised bioconcentration test design (48 h uptake, 48 h depuration) was applied to several taxa spanning multiple phyla and a broad size range under controlled laboratory conditions. Concentrations of carbamazepine were determined in the water phase and animal tissue at the end of the uptake and depuration phases. Toxicokinetic parameters (K_{in} and K_{out}) were estimated using a one-compartment first-order model and used to estimate bioconcentration factors. Uptake and depuration rates and BCFs were compared with trait data, compiled from direct morphometric measurements and established ecological trait databases, to identify potential drivers of uptake.

Preliminary analyses indicate that variation in toxicokinetic parameters aligns with key biological traits, such as the surface area-to-volume ratio and lipid content, suggesting that both structural and functional characteristics

influence chemical uptake and elimination. These patterns form the basis for expanding the approach to a broader set of organic chemicals spanning a range of Kow values and including ionisable compounds.

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3.01.P-Mo147 In Silico Modelling of Bioaccumulation of Per- and Polyfluoroalkyl Substances

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Per- and polyfluoroalkyl substances (PFAS) are a large family of fluorinated chemicals that vary widely in their chemical and physical properties. The persistence and mobility of certain PFAS, combined with their extensive use over decades in industrial applications and consumer products, have led to their global environmental presence. Toxicological studies have raised concerns about the bioaccumulative nature and potential health risks associated with some PFAS. However, risk assessment remains challenging due to their unique physicochemical properties, which result in complex and poorly understood biological and chemical transformation pathways. To address these challenges, developing non-animal testing approaches to evaluate the environmental fate and health-related properties of PFAS is essential. In this study, a new quantitative structure-activity relationship (QSAR) model has been developed for predicting the bioconcentration factor (BCF) of PFAS. Two datasets were built to investigate bioconcentration processes of PFAS. The first includes experimentally derived BCF values obtained from various open sources, curated to include only fish BCF data generated under the OECD 305 test guideline. The second dataset comprises documented *in vivo* and *in vitro* metabolism extracted from publicly available databases. This metabolism dataset was used to identify key molecular transformations and to train the system to simulate fish liver metabolism. A review of the literature indicates that PFAS bioaccumulation occurs through both passive diffusion (driven by lipophilicity) and active transport (mediated by protein interactions). These two mechanisms were used to derive the maximum bioconcentration potential (BCFMAX) of PFAS. Subsequently, several mitigating factors, such as molecular size, metabolic transformation of the parent compound, water solubility, and ionization, were incorporated to reduce the BCFMAX values. The resulting model demonstrates strong predictive performance for both endpoint estimation and simulated metabolism. It correctly predicts 91% of the chemicals in the training set within the experimental error and exhibits an average sensitivity of over 95% with respect to metabolism simulation.

3.01.P-Mo148 Practical Challenges, Methodological Insights and Key Takeaways from Conducting a *Hyalella azteca* Bioconcentration Pilot Study According to the OECD Test Guideline 321

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Bioaccumulation testing is an important component in assessing a chemical's impact on the environment and human health as well as a conditional requirement in the registration process of modern chemicals. OECD Test Guideline 305 "Bioaccumulation in Fish" has undergone multiple revisions; however, new bioaccumulation test guidelines were not approved until 2024 when the OECD 321 guideline for the "*Hyalella azteca* Bioconcentration Test" (HYBIT) was adopted. This new guideline and test design provides the option for an invertebrate bioaccumulation test in an aqueous exposure system, which is the preferred test for bioaccumulation under REACH. To evaluate feasibility and refine laboratory procedures, a pilot bioconcentration study was conducted in our contract research organization (CRO) laboratory in accordance with the newly adopted OECD 321 guideline. This poster summarizes the challenges, insights gained and key lessons learned from the CRO perspective providing practical considerations for future studies used for regulatory submissions.

3.01.P-Mo149 OECD Ring Trial involving the NanoHYBIT: A Novel Method for Non-Vertebrate Regulatory Bioaccumulation Testing of Nanomaterials

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Validated non-animal methods for the regulatory bioaccumulation assessment, adapted to the specific needs for investigating engineered nanomaterials (ENMs) are still lacking in current guidelines. The freshwater amphipod

Hyaella azteca has proven suitable for determining bioconcentration (BCF) and biomagnification (BMF) factors for ENMs, and initial protocols have been established. These were further refined and standardized within the BMFTR-funded projects NanoHYBIT (FKZ 16LW0289) and NanoHYBIT 2.0 (FKZ 03LW0729), expanding the dataset for various ENMs. In conclusion, the resulting *Hyaella azteca* Nanomaterial Bioaccumulation Test (NanoHYBIT) shows strong potential as a non-vertebrate alternative for bioconcentration and biomagnification studies.

Before regulatory acceptance, however, validation is required to assess protocol transferability across laboratories, evaluation of the assay performance and confirm the reliability and reproducibility of NanoHYBIT-based BCF and BMF estimates for ENMs. Consequently, the OECD Working Party on Manufactured Nanomaterials has recommended validating a protocol aligned with OECD TG 321 through an international OECD ring trial.

Within this ring test, selected ENMs will be assessed for bioavailability and bioaccumulation (BCF and BMF), covering key properties influencing uptake and bioaccumulation. These include dispersibility versus agglomeration behavior, ion release, regulated uptake mechanisms, and the physiological relevance of the metal, chemical stability of metal or metal-oxide or polymer-based ENMs to define the applicability domain of the method.

The multi-laboratory ring trial aims to: i) compare BCF and BMF values generated across participating laboratories, and ii) provide the evidence base required to develop a new OECD Test Guideline for nanomaterial bioconcentration and biomagnification testing using *H. azteca*.

The ultimate goal is to establish a new OECD TG, based on the framework of OECD TG 321, that incorporates both aqueous bioconcentration testing and dietary biomagnification testing with *H. azteca*. This would provide a validated, non-vertebrate method tailored specifically to nanomaterials and ready for regulatory use.

3.01.P-Mo150 Assessing the OECD 321 *Hyaella azteca* Bioconcentration Test (HYBIT) for Polymer-Based Nanomaterials Using 14C-Labeled Polystyrene Nanoparticles

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The identification and scientific evaluation of compounds that bioaccumulate in organisms and biomagnify in food webs is a key element of PBT assessment. Bioaccumulation potential is commonly expressed as bioconcentration factors (BCFs), derived from flow-through fish studies following OECD TG 305. For substances that cannot be maintained under constant aqueous exposure, TG 305 also allows dietary exposure to determine biomagnification factors (BMFs).

Recently, OECD TG 321 introduced a new non-vertebrate test (HYBIT) using the freshwater amphipod *Hyaella azteca* to determine BCF values of soluble, non-particulate substances. Although the OECD GD 406 for assessing the apparent accumulation potential of nanomaterials via dietary exposure in fish tests according OECD TG 305 provides guidance on testing engineered nanomaterials (ENMs), a non-vertebrate approach is desirable in light of the 3Rs principles.

Several studies have shown that *H. azteca* is capable to ingest metal- or metal-oxide-based ENMs present in water, sediment, or diet.

In this study, we evaluated the suitability of the HYBIT for polymer-based ENMs using self-synthesized 14C-labeled polystyrene nanoparticles (14C-nPS).

Besides aqueous exposures, we conducted a dietary exposure test using ENM-enriched artificial food to derive BMF data. Additional experiments assessed potential concentration-dependent uptake and the influence of amphipod sex on ENM accumulation.

Samples collected over 7–21 days were analyzed via liquid scintillation counting to quantify body burden and corresponding water or food concentrations. Using 14C-labeled nanoparticles enables straightforward detection and quantification of the ENMs based on their radioactivity, with minimal to no interference from the sample matrix.

Our results demonstrate that the applied methods are suitable for assessing the bioaccumulation potential of polymer-based ENMs following aqueous and dietary exposure. The resulting BCF and BMF values suggest an overall low bioaccumulation potential of the tested polystyrene ENMs.

3.01.P-Mo151 The Influence of Metal–Organic Mixtures on Chemical Speciation and Bioaccumulation in a Microcosm Study.

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Freshwater ecosystems face growing pressure from complex mixtures of contaminants driven by agricultural practices, urbanization, and shifting climatic regimes. Copper-based products and strobilurin fungicides, such as azoxystrobin, are among the most frequently detected substances in surface waters, often occurring together. Yet, their combined influence on aquatic communities remains poorly understood, as most toxicological information derives from single-stressor laboratory studies on single biological species. This limits understanding of key drivers of ecological change in environments where multiple contaminants interact. The challenge is heightened by the dynamic behavior of copper, whose speciation depends on dissolved organic carbon concentration, pH, ionic strength and other organic compounds in the system. These fluctuations alter bioavailable fractions. Diffusive Gradients in Thin Films (DGT) devices, which provide time-integrated estimates of the chemical species which are (partially) labile on the DGT timescale, offer valuable insight into potential metal bioavailability under variable chemical conditions.

To address these complexities, a three-month microcosm experiment was conducted to explore the combined effects of copper and azoxystrobin on a simplified freshwater ecosystem. Throughout the exposure period, temperature, pH, conductivity, dissolved oxygen, and dissolved organic carbon were monitored alongside repeated DGT deployments to characterize DGT-labile copper in parallel with conventional water sampling. A variety of organisms were collected at multiple intervals during exposure and at the final exposure stage to quantify internal copper concentrations. These samples are currently undergoing processing, and the resulting data will clarify how chemical speciation and DGT-labile metal fractions relate to internal accumulation. This study aims to generate an integrated understanding of how metal-organic mixtures behave under ecologically relevant conditions by linking water chemistry dynamics, chemical speciation processes, and organismal accumulation. The resulting dataset is expected to provide insights into the factors governing the bioavailability and biouptake of contaminants in a mixed exposure scenario in freshwater systems, and to support future toxicokinetic evaluations and ecological risk assessments under increasingly complex pollution scenarios.

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3.01.P-Mo153 Ecological Determinants of Mercury Accumulation in South Atlantic Marine Tetrapods

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Mercury is a persistent toxic metal that biomagnifies in marine food webs, creating risks for wildlife. Understanding how ecological traits of organisms shape mercury bioaccumulation patterns across different groups of marine tetrapods is essential for improving species risk assessment and management planning of their habitats. Here, we investigate the patterns of mercury bioaccumulation in sea turtles, seabirds and cetaceans and their association with organisms' trophic position in the South Atlantic Brazil.

We analyzed mercury concentration in the liver tissue of 151 stranded individuals from 18 species along the Brazilian coast and assessed $\delta^{15}\text{N}$, as a proxy for trophic position, in 93 of those individuals. We evaluated whether mercury concentration increases with trophic level and whether species with similar feeding strategies show comparable bioaccumulation patterns despite taxonomic differences.

Mercury levels varied widely across species and reflected their ecological habits. Sea turtles showed the lowest concentrations, consistent with their low trophic positions. Seabirds displayed a strong ecological variation, with pelagic predators presenting the highest values. Cetaceans showed the broadest variation, from low levels in coastal dolphins to extremely elevated concentrations in deep-diving oceanic species.

Grouping species by similar biological characteristics revealed a clear bioaccumulation pattern: oceanic cetaceans presented the highest values, followed by pelagic seabirds, non-flying seabirds, coastal cetaceans, estuarine birds, turtles and migratory cetaceans. This pattern highlights

intensified biomagnification in oceanic food webs and more homogeneous exposure among coastal species feeding on similar prey. Mercury was positively correlated with $\delta^{15}\text{N}$, confirming trophic-driven accumulation across taxa. Isotopic niches showed clear segregation among groups and broad trophic diversity in cetaceans.

Overall, mercury exposure in marine tetrapods is primarily determined by trophic position, foraging strategy and habitat use. Ecologically similar species accumulated comparable mercury levels regardless of taxonomy, underscoring the importance of multispecies ecological approaches for improving contaminant monitoring in marine ecosystems.

3.01.P-Mo154 Use of Fish In Vitro Clearance Assays for Bioaccumulation Assessment

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ECHA aims to reduce both the use of vertebrate animals and the number of animals required for chemical testing. For bioaccumulation assessments, this could be supported by using fish in vitro intrinsic clearance assays (OECD Test Guideline 319A/B) as an alternative to in vivo bioaccumulation studies (OECD Test Guideline 305). To evaluate this approach for regulatory use, ECHA established an ad hoc expert working group to provide scientific advice on the use of in vitro rainbow trout clearance assays, in vitro-in vivo extrapolation (IVIVE) and bioconcentration factor (BCF) models in bioaccumulation assessment. This advice will be based on an extensive comparison of empirical data and will consider several IVIVE/BCF prediction methods.

As a first step, good quality fish BCF and in vitro clearance assay data were collected for ~100 chemicals spanning a broad chemical space (neutral and ionizable organic chemicals; log KOW < 3 - 8). The data were evaluated against relevant OECD test guideline validity criteria to ensure that they are sufficiently reliable for further analysis.

The latest IVIVE/BCF models from the scientific literature were reviewed, their key input parameters summarized, and similarities/differences between the models assessed. A sensitivity analysis was conducted to assess model performance under different biotransformation scenarios (slow, moderate, fast) at log KOW values between 2 and 10. A subset of the reviewed IVIVE/BCF models will be used to predict BCFs from the fish in vitro clearance rate for the collected chemicals.

Finally, for each chemical, predicted BCFs will be compared with the corresponding experimental in vivo fish BCFs to examine data trends and identify any outliers. This comparison will help clarify the relationship between IVIVE-based predictions and measured in vivo outcomes. Based on this evaluation, we will recommend how the reporting of fish in vitro assay results can be improved and how these data can be integrated into regulatory bioaccumulation assessments.

3.01.P-Mo155 Applicability of fish cell lines for the bioaccumulation and toxicity assessment of structurally diverse zwitterionic surfactants

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Zwitterionic surfactants, widely used in consumer products and frequently detected in aquatic environments, exemplify the difficulties to conduct environmental risk assessment of chemicals with complex physicochemical properties (e.g. ionization, low solubility, and high hydrophobicity). To address the lack of available data regarding their environmental risks and improve the mechanistic understanding of surfactant-organism interactions, we evaluated the applicability of animal-free testing methods, namely the rainbow trout (*Oncorhynchus mykiss*) cell lines RTgill-W1 and RTL-W1, to characterize the acute toxicity and/or bioaccumulation of structurally diverse zwitterionic surfactants differing in carbon-chain length and functional groups. Cell viability assays, following the OECD 249 test guideline, revealed that amine oxide (AO) congeners

with 10-, 12-, and 14-carbon chains exhibited increasing toxicity with chain length (EC50 values: 134, 7, and 2 mg L⁻¹, respectively), though notable bioaccumulation was only observed for the AO-C12 compound (BCF_RTgill-W1: 52, BCF_RTL-W1: 41). In contrast, AO-C16 and AO-C18 congeners showed no further increase in toxicity nor bioaccumulation, suggesting limited bioavailability, likely due to their complex behavior. Furthermore, surfactants containing additional amide and carboxylate functionalities, including cocamidopropyl betaine and (Lauryldimethylammonio)acetate, displayed the highest cell-based bioaccumulation among all tested compounds: BCF_RTgill-W1: 303, BCF_RTL-W1: 254 and BCF_RTgill-W1: 77, BCF_RTL-W1: 133, respectively. Despite these findings, all tested surfactants remained below regulatory bioaccumulation thresholds under REACH. Collectively, these results reinforce the relevance of fish cell line-based assays as robust, animal-free tools to support the environmental risk assessment of structurally diverse zwitterionic surfactants.

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3.01.P-Mo156 Microcystin Accumulation and Progressive Liver Damage in Fish Under Repeated Exposure Conditions

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Fish from eutrophic environments are exposed to recurrent cyanobacterial blooms, yet the biological processes underlying microcystins (MCs) accumulation under repeated exposures remain poorly characterized. The understanding of these processes is essential for improving toxicokinetic–toxicodynamic and ecological models to predict toxin burdens in natural environments. To address this gap, three experiments were conducted using *Microcystis* blooms containing MC-LR as a dominant congener, in triplicate, using 15 individuals (*Australoheros facetus*) per 20 L aquarium. A first experiment examined the kinetics of accumulation and clearance after a single 24-h exposure to a high MCs concentration (600 µg/L), followed by a 7-day depuration period. Two additional experiments simulated recurrent bloom events through repeated exposures at medium (60 µg/L) and high (600 µg/L) MCs concentrations. Blooms were added on day 1 and reintroduced on day 10 after MC levels in water declined to <1–5%. Fish were sampled on day 10 for histopathology and on day 18 for histopathology and MCs content. Analysis of MCs and its detoxification conjugates (n=6 per condition) was performed using our previously developed quantitative MALDI-ToF method with nanobody-based pre-concentration. After the single exposure, fish reached their highest MC accumulation at the end of intoxication, and ~95% of the toxin was cleared by day 7, coinciding with partial recovery of early liver damage. In contrast, repeated exposures produced progressive MC-typical hepatic lesions, more pronounced at 600 µg/L. By day 18, median MC concentrations were 15.8 ± 5.5 ng/g at 60 µg/L and 19.2 ± 21.0 ng/g at 600 µg/L, both exceeding the peak value observed after the single 1-day exposure (5.2 ± 6.5 ng/g). Because small group sizes and high variability limit formal comparisons, only descriptive statistics are presented in this preliminary report. Overall, these results suggest that factors associated with repeated exposure—including incomplete hepatic recovery—may reduce detoxification efficiency and influence toxin retention more strongly than exposure concentration alone. While this mechanism is provisional, it offers a biologically plausible interpretation that may guide more targeted experimental designs and improve ecological models of toxin dynamics in eutrophic fish populations.

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3.01.P-Mo157 Feasibility Assay for OECD 305 Aqueous Exposure Test

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The purpose of the OECD 305 guideline test is to assess the bioaccumulation potential of a test item via aqueous exposure or through a dietary route in fish. The tests are usually conducted with radiolabeled test items and optimum test concentrations are determined from preliminary testing. The use of radiolabeled test item allows assessment of water or feed concentrations to be monitored, accumulation and elimination of the test item to be followed and assess if metabolism of the test item has occurred which may require identification and quantification.

The preferred testing method is an aqueous exposure route and the choice of test is based on many factors including: solubility in water, n-octanol water partition coefficient (KOW), stability in water which is considered along with the regulatory requirements. The aqueous exposure route is most suited to stable organic test items with log KOW values generally between 1.5 and 6.0. Specific guidance cannot be provided on the method to choose for a test item due to other factors that may influence the method applicability. Although it is considered for substances with a log KOW above 5 and a water solubility below ~0.01 – 0.1 mg/L, an aqueous exposure route may be difficult to conduct.

To aid in the reasoning to conduct a dietary study where the test item characteristics may indicate an aqueous study should be conducted and has been requested by a regulatory authority, a feasibility assay has been developed. The test has been established to provide evidence that a stable, measurable dissolved concentration of the test item in water cannot be achieved.

The experiments designed for the feasibility assay use small tests which are devised to establish the solubility and stability of the test item in a dose solution and a small portion of test water at a nominal exposure concentration. The outcome of these tests supports whether a stable, measurable dissolved concentration of the test item in water can or cannot be achieved and helps define which testing method should be used for the OECD 305 study.

The conduct of the feasibility assay has shown to aid in the submission process of the test item for the client as weight of scientific evidence for justification to perform a dietary exposure test.

The poster will present an experimental design with the two different possible outcomes that can be obtained: aqueous exposure route being viable or a dietary exposure route.

3.01.P-Mo158 Do polycyclic aromatic hydrocarbons exhibit oral bioaccumulation in aquatic organisms? – Evaluations using fish and shellfish

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Aquatic organisms can absorb chemicals from the various tissues, with the primary routes are considered to be the uptake of waterborne chemicals through the respiration via the gill, and ingestion of ones contained in the diet during feeding. In the previous studies, there has been much information for accumulations of chemicals absorbed through the respiration and, as a result, bioconcentration factors (BCFs) estimated from the exposure examinations are frequently shown.

We previously conducted oral exposure to several polycyclic aromatic hydrocarbons (PAHs) in a few marine fish species. While the internal transfer of PAHs was confirmed, accumulation relative to the concentrations in the feed was not observed. Since then, we have examined multiple times whether PAHs could undergo oral bioaccumulation in fish, but we have not been able to confirm it.

In the present study, we used common carp (*Cyprinus carpio*), which is widely employed in chemical substance assessments in Japan, to newly perform oral bioaccumulation tests on several PAHs and verify whether accumulation can be detected. In parallel, we also conducted water-exposure/gill-uptake tests and compared the results.

The target PAHs, with logKow values ranging from 4.5 to 7.1, were dibenzothiophene, phenanthrene, methylphenanthrene, anthracene, pyrene, benz(a)anthracene, chrysene, benzo(a)pyrene, benzo(k)fluoranthene, and dibenz(a,h)anthracene. Each compound was added to the feed to achieve concentrations of 1 mg/g and 10 mg/g, and exposure was continued for 28 days. As a result, transfer from feed to tissues was observed for some PAHs, such as phenanthrene and pyrene; however, no accumulation with biomagnification factors exceeding 1 was observed. In contrast, when water exposure was conducted, a certain degree of bioconcentration was observed, with bioconcentration factors reaching several tens to several hundred. These results suggest that PAH accumulation in fish depends predominantly on gill-mediated bioconcentration, and the contribution of oral bioaccumulation is considered to be extremely low.

In this study, in addition to the results of the verification using carp, we conducted oral bioaccumulation tests of PAHs in the gastropod *Batillaria multiformis* (the estuarine snail) and report the findings on their accumulation levels as well.

3.01.P-Mo159 Bioaccumulation and Depuration of Lithium in Juvenile Trout (*Oncorhynchus mykiss*): A Comparative Study of Waterborne and Dietary Exposure Pathways

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Lithium (Li), an emerging contaminant, has attracted increasing attention due to its high application in battery production, pharmaceuticals, among others, combined its potential toxicity in aquatic systems. To simulate environmentally relevant exposure routes, we conducted three experiments using juvenile trout (*Oncorhynchus mykiss*) as a model organism: (1) waterborne exposure, (2) dietary exposure, and (3) depuration following waterborne exposure. Time-resolved tissue sampling and tissue Li was used to evaluate Li bioaccumulation and depuration dynamics, and Li isotope ratios will also be investigated.

In the waterborne exposure experiment, four Li concentrations (control, 0.1, 1.0, and 10 mg/L) were tested, with three replicates per treatment. Fish were exposed for 4 days and samples were collected at 0.25, 1, 2, and 4 days. At 10 mg/L acute toxic effects were observed within 24 hours, with 27% mortality recorded by 48 hours, leading to early termination of this experiment. In the remaining treatments, Li accumulation was time dependent. By day 4, tissue Li concentrations reached 1.74 ± 0.50 and $42.1 \pm 12.2 \mu\text{g/g}_{\text{dw}}$ in the 0.1 and 1.0 mg/L treatments, respectively. Higher exposure concentrations resulted in faster accumulation rates and increased concentration factors, indicating nonlinear uptake behavior.

In the dietary exposure experiment, fish were fed a single dose (3% of body weight) of food pellets containing 5.8 $\mu\text{g/kg}$ Li, with sampling at 0, 3, 6, 24, and 48 hours ($n = 3$). Li was detectable in fish between 3–6 hours post-feeding but returned to control concentration levels after 24 hours, indicating limited Li body retention and rapid elimination.

In the depuration experiment, fish pre-exposed to 0.1 $\mu\text{g/kg}$ Li for 48 hours were transferred to an open clean-water system and sampled at 0, 3, 6, 24, and 48 hours ($n = 3$). Li concentrations in tissues declined rapidly and returned to control levels within 24 hours, mirroring the rapid elimination observed in the dietary exposure experiment.

This study highlights the route-dependent bioaccumulation and elimination dynamics of lithium in juvenile trout (*Oncorhynchus mykiss*) and provides experimental evidence to inform mechanistic insights and risk assessment related to Li contamination in aquatic environment.

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3.01.P-Mo160 Species-specific Accumulation and Body Distribution of Organophosphate Esters and Their Metabolites in Marine Mammals along the Korean Coast

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Organophosphate esters (OPEs) are widely used as flame retardants and plasticizers following restrictions on brominated flame retardants. OPEs can be transformed into organophosphate diesters (di-OPEs) and hydroxylated OPEs (OH-OPEs), some of which exhibit higher toxicity or bioaccumulation potential than their parent compounds. Information on environmental and biological occurrences of OPE metabolites (mOPEs), particularly OH-OPEs, remains limited.

Marine mammals serve as sentinel species for evaluating marine ecosystem health, yet only a few studies have investigated OPEs and mOPEs in their tissues, and tissue-specific accumulation and metabolic patterns remain poorly understood.

In this study, muscle, liver, kidney, heart, lung, intestine, stomach, blubber, melon, and brain were collected from eight cetaceans and two pinnipeds incidentally captured or stranded along the Korean coast between 2008 and 2019. Eighteen OPEs, ten di-OPEs, and six OH-OPEs were quantified using ultrahigh-performance liquid chromatography coupled with triple quadrupole mass spectrometry.

Among parent OPEs, alkyl-OPEs such as tris(2-butoxyethyl) phosphate (TBOEP), tri-n-butyl phosphate (TnBP), and triethyl phosphate (TEP) dominated across tissues. For mOPEs, OH-OPEs, including hydroxyphenyl phenyl hydrogen phosphate (OH-TPHP) and hydroxyethyl bis(2-butoxyethyl) phosphate

(BBOEHEP) were frequently detected at higher levels than di-OPEs, underscoring the need to examine OH-OPEs in environmental and biological monitoring. Both OPEs and mOPEs, such as TBOEP, TnBP, TEP, diethyl phosphate (DEP), and OH-TPHP, were detected in brain tissues, suggesting that not only OPEs but also metabolites may cross the blood–brain barrier.

Across tissues, metabolites of TBOEP, TnBP, triphenyl phosphate (TPHP), and tris(2-ethylhexyl) phosphate (TEHP) showed higher proportions than their parent compounds in long-term storage tissues such as muscle and blubber, indicating bioaccumulation potential of mOPEs. Some species, particularly fin whales, exhibited higher proportions of parent OPEs relative to metabolites, implying possible interspecies differences in metabolic capacity.

The widespread detection of mOPEs, especially OH-OPEs, in diverse organs highlights their systemic transport and bioaccumulation potential in marine mammals. These findings emphasize the necessity of incorporating mOPEs into future monitoring frameworks and ecological risk assessments.

Disclaimer/Disclosure:

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3.01.P-Mo161 Dioxins in Czech Rivers: Long-Term Monitoring of Fish and Sediments

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Dioxins are persistent organic pollutants characterized by high toxicity, hydrophobicity and a tendency to accumulate in biotic and abiotic environmental compartments. To evaluate the occurrence of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs), we analyzed their levels and distribution in fish and sediments. In total, more than 250 samples of common chub and sediment were collected from major river sampling sites across the Czech Republic during 2014–2025. Results indicated different distribution patterns between the matrices. Throughout the monitoring period, DL-PCBs in fish were above the limit of quantification (LOQ) in 100% of the samples, whereas their occurrence in sediments was generally lower. Conversely, PCDDs and PCDFs were more frequently detected in sediment samples than in fish. On average, PCB 126 was the predominant congener in fish samples (80% of total TEQ), while the concentrations of individual compounds in sediments were more site-dependent. Based on these findings, monitoring of various matrices is needed to comprehensively understand contaminant fate and to appropriately assess aquatic ecosystem contamination.

3.01.P-Mo162 Bioaccumulation, Depletion and Maternal Transfer of Antibiotics in *Eisenia andrei*

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The continuous use of antibiotics in agriculture can affect organisms that are fundamental to maintaining soil health. This study assessed the bioaccumulation, depletion, and reproductive effects of tetracycline (TTC) and sulfamethazine (SMZ) in *Eisenia andrei*, comparing their kinetic behaviour and reproductive toxicity potential under environmentally realistic conditions (TTC; 6.4 ng g⁻¹ e SMZ; 20.4 ng g⁻¹). The assays followed OECD Guideline No. 317, with 21 days of exposure and 21 days of depletion in clean soil, using radiolabelled compounds with quantification performed by liquid scintillation counting.

Both antibiotics exhibited low bioaccumulation (<1% of the applied dose). TTC showed faster elimination ($K_e = 1.3 \times 10^{-1} \text{ day}^{-1}$; $t_{1/2} = 5.3$ days), whereas SMZ exhibited slower elimination ($K_e = 8.2 \times 10^{-2} \text{ day}^{-1}$; $t_{1/2} = 8.5$ days). Regarding reproductive effects, TTC did not differ from the control ($p > 0.97$), while SMZ significantly reduced cocoon production during exposure (ANOVA: $F_{2,9} = 11.59$; $p = 0.0032$; estimate = -11.0; $p = 0.004$) and depletion (ANOVA: $F_{2,9} = 17.14$; $p = 0.00085$; estimate = -13.75; $p = 0.001$). Mean values decreased from 58.8 ± 2.6 (control) to 47.8 ± 2.5 and subsequently to 43.2 ± 1.3 cocoons in the SMZ group.

During bioaccumulation, both antibiotics were transferred to the cocoons (ANOVA: $F_{2,9} = 232.2$; $p < 0.0001$). After depletion, only SMZ retained detectable radioactivity (102 ± 2.4 DPM). This value is close to the method's background level (~100 DPM), yet still above what would be expected for complete elimination, indicating persistence in maternal tissue or delayed release. The control and TTC returned exactly to baseline (all values = 0). The differences remained highly significant (ANOVA: $F_{2,9} = 6936$; $p < 0.0001$), with an effect observed only for SMZ (Dunnett $p < 1 \times 10^{-10}$).

The results show that SMZ exerts substantial and persistent reproductive effects, with a tendency for transfer to cocoons and a potential transgenerational risk, even after the depletion phase. TTC showed rapid clearance and no reproductive effects. These findings reinforce that antibiotics from different classes pose distinct ecological

risks and highlight the need to integrate reproductive endpoints and internal kinetic parameters into environmental risk assessments for agricultural soils.

Disclaimer/Disclosure:

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3.01.P-Mo163 Evaluation of a Non-Vertebrate Approach for the Assessment of Terrestrial Bioaccumulation of 14C-labeled Nanoparticles using Porcellio scaber

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Identifying compounds that bioaccumulate in organisms and biomagnify in food webs is a central aspect of PBT assessment. Bioaccumulation potential is usually expressed as a bioconcentration factor (BCF), determined in flow-through fish studies following OECD TG 305.

For substances that cannot be maintained under stable aqueous exposure conditions, TG 305 also provides a dietary exposure approach to determine biomagnification factors (BMFs). However, this fish test was originally developed for soluble, non-particulate substances. Since 2025, an OECD Guidance Document (No. 406) has been available offering recommendations on how to conduct biomagnification tests with engineered nanomaterials (ENMs). Nevertheless, appropriate non-vertebrate approaches are still missing.

While the *Hyalella azteca* bioconcentration test is currently being evaluated for its suitability as a non-vertebrate alternative for aquatic bioconcentration and biomagnification testing of ENMs, there is still a notable lack of terrestrial test systems that can generate regulatory-relevant endpoints - such as BMFs - with clearly defined uptake routes. For polymer-based nanomaterials (P-NMs) further challenges arise. For instance, conventional analytical approaches often risk artefacts or limitations, such as fluorescence dye leakage from the polymer matrix or altered material properties caused by metal doping for detection and quantification, which may change the physical behavior and environmental fate of P-NMs.

As an initial attempt to evaluate a terrestrial biomagnification test (in soil-free systems) with the isopod *Porcellio scaber* we conducted a feeding experiment with uptake and subsequent depuration phase, to derive kinetic data on P-NM uptake and potential biomagnification.

Radioanalytical methods using synthesized 14C-labeled P-NMs which were embedded in the experimental diet were applied. This allowed a straightforward, sensitive, and artefact-free quantification of dietary exposure, body and feces burdens, and compartment-specific distribution in the isopods.

3.01.P-Mo164 Pesticide Analysis of Water Sources via Solid Phase Microextraction and Ambient Mass Spectrometry

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The widespread use of synthetic pesticides has resulted in environmental accumulation with residues and transformation products being frequently detected in surface and seawater. These contaminants pose a severe risk to the ecosystem and human health; yet current monitoring approaches rely on labour-intensive sampling, followed by off-site laboratory sample preparation and analysis, which limits throughput.

This investigation aims to develop a sensitive, quick, and sustainable method for detecting pesticide contaminants in various water sources using solid-phase microextraction (SPME) coupled with atmospheric solids analysis probe-mass spectrometry (ASAP-MS). To manage the challenges of low-level detection limits and potential matrix contamination, SPME utilises a pre-concentration and clean-up step, enabling efficient analyte transfer from the SPME fibre to the MS inlet while minimising solvent use.

The SPME method involves fibre conditioning, analyte extraction from water samples, and fibre washing using minute reagent volumes. This is followed by direct desorption into the ASAP-MS sample inlet. Mass Spectrometric analysis was carried out in scan and single ion monitoring (SIM) modes under optimised instrument conditions.

The method achieved limits of detection (LoD) in the parts-per-billion (ppb) range, with 11 of the 14 pesticides being successfully detected in either positive mode, negative mode or both. The results suggest that SPME-ASAP-MS provides a fast, sensitive and sustainable technique for pesticide screening in water, particularly seawater, which contains a wide variety of salts, with reduced sample preparation time and reagent consumption compared to conventional workflows. This methodology offers an updated, sustainable approach to environmental monitoring and proposes a field-deployable option for pesticide contamination monitoring in rivers and coastal systems.

3.01.P-Mo165 METABOLIC ALTERATIONS IN FISH EXPOSED TO ACID RESIN CHARACTERIZED BY METABOLOMIC ANALYSIS AND BIOCHEMICAL ANALYSES

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Acid resins are diterpenoids produced by conifers, such as Pinus. These resins are widely released into soil in areas used for Pinus plantations. Pulp and paper industries, when producing cellulosic pulps, generate large quantities of effluents as byproducts, which may contain, on average, 50 to 250 µg/L of resins. Both resins released into soils and those produced in effluents can be carried to water bodies, contaminating them. In this study, the possible deleterious effects of abietic acid, one of the acid resins, were evaluated in fish (*Oreochromis niloticus*), under laboratory conditions, exposed to concentrations in the range of 100 to 150 µg/L. Fish were kept in groups of 10 individuals in 200L tanks and fed with commercial feed. Blood, liver, gonad, and gill samples were collected after a 7-day exposure period, with daily water renewal in the tanks. The obtained blood plasma was analyzed regarding AST, ALT, alkaline phosphatase, total cholesterol, glucose, albumin, and total proteins. Gonads, liver, and gills were homogenized, extracted with solvent (MTBE), and analyzed by GC/MS-TOF, in addition to the analysis of total carbohydrates and lipids. The obtained results indicated significant alterations in the total concentrations of lipids and carbohydrates in the gonads and liver, as well as alterations in the lipid (fatty acids and cholesterol) and carbohydrate profiles analyzed by GC/MS. The level of plasma enzymes was considerably altered, with a significant increase in the activities of the enzymes ALT, AST, and alkaline phosphatase. A significant reduction in plasma glucose and an increase in cholesterol was observed. Total proteins and albumin showed no modifications. Abietic acid proved to be toxic to the analyzed fish, promoting significant metabolic alterations.

3.02.A Advances in Exposure Modelling: Closing the Gap between Scientific Innovation and Regulatory Relevance

3.02.A.T-01 Predicting Chemical Co-Exposure in Europe: Development of a Continental Fate Model

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The ENCORE project develops a probabilistic modelling framework to identify priority chemicals contributing to co-exposure risk across Europe. Central to this effort is the ENCORE fate model, a continental-scale system that estimates environmental concentrations for a broad range of substances, including pesticides, pharmaceuticals, REACH chemicals, and legacy pollutants. The model integrates harmonised input data, advanced emission modelling, hydrological connectivity, and Monte Carlo simulation to account for uncertainty.

The European landscape is discretised into ~23 000 hydrologically connected sub-catchments. Chemical concentrations in surface waters are predicted in two steps. First, emissions are simulated based on substance-specific sources and pathways. Pesticides are modelled via agricultural applications on crops, pharmaceuticals through household consumption and wastewater release, and industrial chemicals via manufacturing and downstream uses. These pathways define the mass of each substance entering the aquatic environment per sub-catchment. Second, the water quality module propagates emissions through the river network while accounting for dilution, partitioning, degradation, and downstream transport.

Input data include application rates, sales and consumption records, and tonnage information for European countries, as well as physicochemical parameters required to simulate environmental fate. Uncertainty in emissions and fate parameters is addressed through Monte Carlo simulations. Preliminary findings show that emissions and source contributions dominate the variability in predicted environmental concentrations, whereas fate parameters exert comparatively less influence.

Model outputs reproduce realistic spatial trends across Europe. For example, predicted diuron concentrations are elevated along major river systems such as the Rhine, Danube and Po, reflecting agricultural use and downstream transport. In contrast, concentrations remain low in Scandinavia and high-altitude Alpine and Balkan regions due to limited agricultural inputs and strong dilution effects.

The ENCORE fate model provides a robust framework for predicting chemical concentration distributions and co-exposure patterns across European freshwater systems. Ongoing work focuses on refining uncertainty analyses and extending the framework to probabilistic mixture risk assessment, including the influence of legacy chemicals and variability in hazard data.

Disclaimer/Disclosure:

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3.02.A.T-02 Spatially Referenced Environmental Exposure Model for Down-the-drain Substance Emissions Across European Rivers

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A key aspect of chemical environmental safety assessment is understanding the environmental fate, transport, and exposure of a chemical. For ingredients used in household products that go down-the-drain, exposure assessment focuses on receiving waters from municipal waste water treatment plants. Traditionally, exposure assessment frameworks represent the environment as a single area using a deterministic approach and does not account for the spatial variability across a given country or region. To address this gap, we have developed a global framework based on the iSTREEM spatial modeling framework, to incorporate spatial variability in key determinants of down-the-drain substance environmental exposures. In this presentation, we focus on the expansion of the model to European rivers including the 27 EU member states, Norway, Switzerland, and the United Kingdom. Model inputs combine European Union data on wastewater-treatment plants, their locations and infrastructure, with global spatial datasets on population, river discharge, and routing. The model outputs concentration distributions that reflect geographic variation in these parameters while incorporating river connectivity, chemical movement between rivers, and in-stream decay. Chemical-specific inputs comprise wastewater treatment removals, in-stream decay rates, and emission estimates.

Four case-study chemicals that are commonly used in consumer products with down-the-drain exposure pathways were used to demonstrate the approach and evaluate model predictions: linear alkylbenzene sulfonates (LAS) and alkyl sulfates (AS) as surfactants used in products such as laundry detergents, and oxybenzone and octinoxate as UV-filter ingredients used in personal care products. Monitoring data were collected from several sources including European monitoring databases and the literature and compared to modelled concentrations. The results show that modelled concentrations are both predictive and conservative, with the 90th percentile of modelled values aligning with observed concentrations within a factor of two to eight across the four substances. The study demonstrates how the model can be used in prospective safety assessments by providing a tool to aid in understanding of chemical concentrations accounting for spatial variability in key aquatic exposure parameters.

3.02.A.T-03 Bridging Innovation and Regulation: High-Resolution Spatial Mapping of Sewage Connectivity for Refined Exposure Modelling

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Chemicals from home and personal care products often enter aquatic environments via wastewater. Sewage treatment plants (STPs) play a critical role in removing these down-the-drain chemicals before their release into the aquatic environment. The connectivity rate, which defines the proportion of sewage safely treated, is key for environmental exposure modeling and risk assessment. However, existing estimates of connectivity rely

primarily on large-scale national surveys and coarse regional statistics, resulting in a lack of spatial resolution. In this case study for China, we developed a mathematical model to estimate STP connectivity, including a sewershed-based approach followed by a moving-window method. Based on these methods, we allocated gridded population cells to each STP and summarized connectivity at multiple spatial levels. We optimized the parameters using diverse data sources and subsequently generated a high-resolution (1-km) connectivity dataset. Under the most realistic scenario, the calculated Chinese national average sewage treatment connectivity was 46%, consistent with their regulatory default value (50%). Significant regional differences emerged with STP connectivity ranging from 0.4% to 99.9%. By using the moving window method to smooth the influence of extreme values and highly populated areas, we obtained a more representative and homogeneous national average of 34%. A comprehensive sensitivity uncertainty analysis was conducted ensuring the robustness of the estimates. Our methodology achieved higher-resolution estimates and improved spatial correspondence through its flexible parameter settings and strong correlation with population distribution, enabling more precise spatial analysis. The refined connectivity methodology can be applied across other countries and regions with results enabling higher-tier down-the-drain emission and exposure modeling, offering a robust scientific basis for regulatory policy-making.

3.02.A.T-04 From Pharmaceutical Emission Modelling Approach Toward an Applicable Risk Assessment Plugin in QGIS

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Pharmaceuticals enter the environment primarily via wastewater treatment plants (WWTPs). Assessing environmental risks from pharmaceuticals is challenging due to the limited availability of monitoring data, particularly in rural areas and at small to mid-sized WWTPs. While awareness is increasing and introduced into EU legislation by the European Urban Wastewater Treatment Directive (UWWTD) in January 2025, data gaps persist.

Currently there is no unified risk assessment methodology to be used under the UWWTD. Such a methodology is needed to ensure the comparability of risk assessment results between river basins and countries. Based on established scientific approaches in exposure modelling, a deterministic steady-state-model was developed in an open-source QGIS plugin.

The approach integrates yearly pharmaceutical sales statistics and WWTP monitoring data to estimate active pharmaceutical ingredient (API) loads. Transferred to the river network, predicted environmental concentrations (PECs) were calculated and compared to available monitoring data. Flow was implemented as spatially distributed yearly averages in subcatchments. Dividing PECs by predicted no-effect concentration (PNECs) resulted in risk quotients (RQs) that were then mapped for river sections in catchments. These result maps directly support national and regional authorities in charge of risk assessment. Using this model, decision-makers can directly prioritize WWTPs between 10,000 and 150,000 population equivalents (PE) for quaternary treatment according to UWWTD in their daily-work practice in QGIS. In addition, other feasible mitigation options for smaller WWTPs can be tested *a priori*.

In this study, we tested the plugin in the German Warnow catchment as well as in the Finnish Kyrönjoki catchment. Modelled and monitored API loads were compared for selected APIs, such as Diclofenac. Results showed that PECs aligned well with averages from campaigns conducted in the catchments. To improve model accuracy, estimations of WWTP discharge loads were corrected by monitored data. Finally, the model showed that upgrading the mid-sized WWTPs will have only a limited effect in the rural areas with scattered treatment, especially upstream.

With its high relevance and applicability for regulation, the developed plugin is a user-friendly tool for authorities to close the gap towards regulatory practices: users can identify potential risk areas by integrating available expertise and new monitoring data.

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3.02.A.T-05 An LLM-based Toolbox for Automated Text Mining on the Uses of Chemicals

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The large number of chemicals used globally and the limited availability of structured information on their applications create challenges for exposure estimation, in silico modeling, and chemical safety assessment. Information on how chemicals are used is distributed across scientific literature, regulatory submissions, and technical reports, which makes it difficult to assemble coherent datasets for environmental and human health evaluations.

To address this challenge, we developed an open-access and fully automated toolbox that curates, standardizes, and integrates chemical use information at scale. The workflow applies a two-stage natural language processing framework based on large language models to extract chemical use pairs from unstructured text. The first stage is fine-tuned using low-rank adaptation, which allows the model to identify text segments that contain potential chemical information. The second stage uses a fine-tuned large language model trained with direct preference optimization to improve extraction accuracy for environmentally relevant chemical terminology. The toolbox includes components for named entity recognition, text classification, and relationship extraction. These components identify chemical entities, assign functional use categories, and organize results into structured formats that can be linked with existing datasets.

By applying this toolbox, we aim to develop an open access resource that compiles and standardizes chemical and material use data. The resulting dataset is intended to support chemical assessment, increase transparency, and provide a foundational resource for regulatory and research activities. Future work will focus on improving model performance, expanding data sources, and refining ontological alignment. These developments aim to increase the reliability and applicability of extracted information and to facilitate broader use of the toolbox within chemical safety and data harmonisation initiatives.

3.02.B Advances in Exposure Modelling: Closing the Gap between Scientific Innovation and Regulatory Relevance

3.02.B.T-01 Modeling Environmental Plastic Emissions: A Particle-based Approach to Dynamic Probabilistic Material Flow Analysis

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Macro- and microplastic emission, transport, and fate models require that material characteristics such as size and shape are addressed, rather than focusing solely on mass flows. Existing dynamic probabilistic material flow analysis (DPMFA) models provide mass-based estimates but do not resolve particle size or shape, limiting realism in environmental fate and exposure assessments based on these models. Here, we introduce the use of size-dependent transfer coefficients in MFA modeling, enabling the partitioning of plastic flows while accounting for particle size distributions and cutoff sizes, thereby producing distinct, realistic emission pathways. Our novel DPMFA plastic emission model distinguishes between in-use and end-of-life emissions, capturing the temporal dynamics and differing emission potentials of (micro)plastics generated during use and (macro)plastics entering waste streams. We applied the model to the Netherlands and simulated plastic flows and emissions from 1950 to 2050. We show that using size-dependent transfer coefficients (SDTCs) not only leads to lower plastic emissions to some environmental compartments but also results in different allocations of plastics across compartments compared to traditional mass-based DPMFA models. Finally, we generated the size, shape, and density distributions of the polydisperse plastic particle mixtures emitted into the Dutch environment. Our DPMFA plastic emission model provides a robust tool to assess how policy measures targeting plastic production and use influence environmental emissions and associated risks.

3.02.B.T-02 Quantifying and Mitigating Environmental Emissions and Exposure of Intentionally Added Microplastics in the UK

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Microplastics are recognised as a widespread and persistent pollutant with potentially serious environmental and health impacts. They originate either as “intentionally added” microplastics (IAMP), added to products like cosmetics, detergents and paints, or as secondary microplastics formed through the breakdown of larger plastic items. While emissions from secondary microplastics are hard to regulate, IAMPs offer clearer opportunities for targeted regulatory intervention.

This study provides the first unified UK assessment of IAMP emissions and exposure across environmental compartments, with consideration of future risk management options. A framework was developed that reconstructed historical emissions (pre 2024) using sector-specific starting years and diffusion-based interpolation, and predicted future projections (2024–2043) based on UK market data, PRODCOM datasets, expert judgment, and scaled EU values. Material flow analysis mapped emission pathways and environmental exposure was simulated using a dynamic version of the UTOPIA microplastic unit-world exposure model, covering 17 compartments and accounting for particle size, transformations, and multimedia transport.

Results for the baseline “business-as-usual” scenario show soils as the dominant receiving environment, particularly agricultural soils via biosolid application, since wastewater treatment partitions ~99% of microplastics to biosolids, and almost all biosolids are applied to agricultural land in the UK. Urban soils are the second-largest sink, driven by runoff of infill from synthetic sports surfaces. Among sectors, synthetic sports infill contributes the highest emissions and exposure, followed by detergents.

Risk management scenarios highlight that broad restrictions with few derogations achieve the largest reductions in exposure. Targeted measures, such as improved controls for synthetic sports pitches or limits on biosolid application, are effective in specific compartments but less effective overall.

This work demonstrates the importance of considering both emission and exposure dynamics when prioritising regulatory interventions, showing how such work can feed into evidence-based regulatory decision-making.

3.02.B.T-03 A Modular Fate and Transport Model for Biological Treatment of Micropollutants During Wastewater Treatment

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Sewage treatment plants (STPs) play a critical role in reducing micropollutant emissions and discharges to the environment, yet comprehensive monitoring remains costly and difficult. We introduce a modular fate and transport model, developed in Python, to simulate the behaviour and treatment of organic micropollutants across various STP configurations. The model uses a multimedia box approach for STP compartments and combines mass-balance equations for the activated sludge process, the trickling filter, and the membrane bioreactor and an optional quaternary treatment using granular activated carbon. The framework requires only a small set of physico-chemical properties and key operating parameters to estimate emissions to air, water, and solids. Validation and calibration were performed using influent and effluent data for 40 emerging contaminants from 71 UK STPs (UK Chemical Investigations Programme; over 40000 data points), with performance assessed by MAE, MSE, and RMSE. The modular model's outputs are compared with results from SimpleTreat and TOXCHEM, which used the same dataset for validation. Previous comparisons between SimpleTreat and TOXCHEM showed that predicted removal efficiencies are more affected by chemical properties than plant operating conditions, and both tend to overestimate sorption and volatilisation, especially for per- and polyfluoroalkyl substances (PFAS). This new framework offers a transparent, reusable, and easily extendable environment to support regulators in scenario analysis, process selection, and future integration of data-driven models for recalcitrant and emerging contaminants. Its modular design enables replacement of treatment processes, multiple scenario analyses, and expansion as regulatory standards evolve (e.g., EU Urban Wastewater Directive).

3.02.B.T-04 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.

3.02.B.T-05 Screening-level exposure Models to Evaluate the direct Release of Cosmetic Ingredients (MERCi): Development and application to UV filters used in suncare products

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Concerns regarding the environmental risks of UV-filters used in suncare products have grown in recent years. Much of this concern has been driven by their use in products by consumers engaged in outdoor recreational activities, such as swimming, which can facilitate their direct emissions into aquatic systems. Uncertainties in estimating the amount of a UV-filter that might be directly emitted to water, however, represent a continuing challenge, and include the need to quantify the number of users of suncare products, the skin area covered, the concentration of the UV-filter in the product and the extent to which the UV-filter is 'washed-off'. This study aims to address this gap, which includes the development and application of screening-level exposure Models to Evaluate the direct Release of Cosmetic Ingredients (MERCi). The tier 1 and 2 MERCi models – SUNscreen and multi-SUNscreen – are described, with application to octocrylene, a well-studied UV-filter used in suncare products. Model results suggest that both tools provide reasonable estimates of environmental exposure. Specifically, the tier 1 SUNscreen model, estimates an environmental concentration of 1690 ng·L⁻¹ in freshwater and range from 765 – 1710 ng·L⁻¹ in marine water capturing differences between low – moderate tidal dilution rates, whereas sediment concentrations are estimated to be 825 ng·g⁻¹ dw (freshwater) and 374 – 838 ng·g⁻¹ dw (marine). The tier 2 multi-SUNscreen model, on the other hand, estimates freshwater concentrations of 324 ng·L⁻¹ in water and 2.04 ng·g⁻¹ dw in sediment, results that are observed to be in good agreement with monitoring data. Overall, both models support a conservative, screening-level approach for estimating environmental exposure to UV-filters, and thus represent tools that can be readily utilized to support both risk assessment and research prioritization.

3.02.P Advances in Exposure Modelling: Closing the Gap between Scientific Innovation and Regulatory Relevance

3.02.P-We177 Validation of SVOC Transfer Modeling from Flooring to Indoor Dust: A Comparative Study of Parameter Determination Scenarios using Test-bed Data

Jong-Hyeon Lee and Jung-Hyun Park, EH R&C Co., Korea, Republic of

Assessing the risk of Semi-Volatile Organic Compounds (SVOCs), particularly phthalates, is crucial for indoor air quality management. Emitted from building materials, SVOCs adsorb onto suspended particles and accumulate in dust due to their low vapor pressure. Consequently, accurately predicting dust concentrations is

essential for exposure assessment. This study evaluates the predictive performance of an existing mechanistic SVOC behavior model using empirical data from a residential test-bed, rather than developing a new model. We utilized a mechanistic model accounting for source emission, diffusion, and dust partitioning. To validate the model, a test-bed was established in an LH experimental housing unit (59?) in Sejong City. Rooms were installed with differing source strengths: PVC flooring (2.65% DEHP) and Deco-tiles (5.39% DEHP). Floor dust samples were collected monthly from July to November 2022. We compared three parameter determination scenarios for calculating the air-dust partition coefficient (K_{dust}) and other variables: 1) site-specific measured variables, 2) literature-based empirical estimates, and 3) theoretical estimates. Field measurements showed dust DEHP concentrations ranged from 96.2 to 997.3 ppm (average 425.6 ppm). The room with higher-content Deco-tiles exhibited the highest concentration (551.6 ppm), confirming a correlation between material content and dust contamination. Simulations revealed significant discrepancies; scenarios using literature estimates yielded highly overestimated concentrations (15,000–23,000 ppm), tens to hundreds of times higher than actual measurements. Conversely, using measured parameters predicted concentrations between 725 and 760 ppm, showing much closer agreement with observed data. This study confirms that model reliability depends critically on parameter accuracy, particularly K_{dust} . Generic literature values led to substantial overestimation of risk. Therefore, applying site-specific measured variables is essential for ensuring the predictive accuracy of exposure assessment models for flooring-derived SVOCs.

Disclaimer/Disclosure:

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3.02.P-We178 Application of an Individual-Based Integrated Exposure Assessment for Predicting Blood Lead Concentrations and Validation Using Biomonitoring Data

Jung-Hyun Park and Jong-Hyeon Lee, EH R&C Co., Korea, Republic of

An individual-based risk assessment for lead exposure was conducted by reflecting children's physiological and behavioral characteristics. Exposure factors such as body weight, inhalation rate, and time spent in various microenvironments were set as fixed values, while the concentrations of lead in indoor and outdoor air, indoor dust and soil, food, drinking water, and children's products were represented by probabilistic distributions. Individual total exposure levels were then estimated using a probabilistic integrated exposure assessment. Food and indoor dust/soil were identified as the dominant exposure pathways for lead, with markedly higher exposure per unit body weight among children under six years of age, indicating greater vulnerability. The integrated exposure estimates were applied to the IEUBK model to predict blood lead concentrations, which were compared with national biomonitoring data. The observed blood lead concentrations were generally higher than those predicted under the average exposure scenario but lower than those under the maximum exposure scenario. The relative contributions of different exposure media varied by exposure level, and analysis of the high-exposure group highlighted the need for targeted and effective exposure-reduction strategies. These results indicate that managing indoor environmental contamination—particularly the control of surface materials and dust in children's activity areas—should be prioritized as a key exposure mitigation strategy. Overall, the findings emphasize the importance of shifting from population-average exposure assessments to individual-based integrated evaluations and developing risk management strategies focused on high-exposure subpopulations for more effective public health protection.

Disclaimer/Disclosure:

This research was supported by the Environmental Technology Development Project (R&D)501 for securing water ecosystem health of the Korea Environment Industry & Technology Institute502 (KEITI). (Ministry of Environment R&D project number: [RS-2020-KE000904])

3.02.P-We179 Improved Chemical Product Evaporation Model (CPEM) using a Top-down Approach: Validation with Liquid Air Fresheners

Jong-Hyeon Lee and Jung-Hyun Park, EH R&C Co., Korea, Republic of

This study improves the Chemical Product Evaporation Model (CPEM) by integrating a "top-down" approach, utilizing measured product consumption rates to overcome the complexity and high uncertainty associated with mass transfer coefficients in traditional "bottom-up" models. We conducted small-scale chamber experiments over a 600-hour period to measure the time-resolved airborne concentrations of D-Limonene and Propylene Glycol emitted from liquid air fresheners. The proposed framework, which employs product weight loss as a primary input constraint, was rigorously compared with four existing mechanistic models—Weschler, Thibodeaux, Langmuir, and AMEM—under various parameter tuning scenarios ranging from complex substance-specific adjustments to a single product-level coefficient. The experimental results confirmed that product mass decreased linearly over time, thereby validating the fundamental assumption of a constant consumption rate (μ) inherent to the top-down approach. While D-Limonene exhibited a rapid initial peak typical of volatile organic compounds, Propylene Glycol showed a gradual concentration increase characteristic of semi-volatile compounds; the improved model successfully captured these distinct behaviors by integrating the total flux constraint with component-specific activity corrections. Notably, while complex tuning scenarios provided high precision, simplifying the model to use a single tuning parameter for the entire product yielded a robust fit to the experimental data, effectively balancing predictive accuracy with model parsimony and avoiding the risk of overfitting observed in multi-parameter cases. By substituting difficult-to-estimate theoretical parameters with easily measurable mass loss data, the improved CPEM significantly reduces predictive uncertainty. Consequently, this hybrid approach offers a practical, scientifically grounded tool for indoor chemical exposure assessment, effectively bridging the gap between theoretical mass transfer principles and real-world consumer product usage patterns.

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This research was supported by the Environmental Technology Development Project (R&D)501 for securing water ecosystem health of the Korea Environment Industry & Technology Institute502 (KEITI). (Ministry of Environment R&D project number: RS-2020-KE000904)

3.02.P-We180 Modelled human exposure to trifluoroacetic acid (TFA) from atmospheric degradation of HFO-1234ze(E) emissions from prospective pMDI usage

Shivendra Tewari¹, Kun Zhao², Krish Vijayaraghavan², Irene Bramke³, Paul Giffen⁴ and Holly Kimko¹, (1)AstraZeneca, United States, (2)Ramboll Americas Engineering Solutions, Inc., United States, (3)AstraZeneca, Netherlands, (4)AstraZeneca, United Kingdom

HFO-1234ze(E) is a near-zero global warming potential, next generation medical propellant, being used in pressurised metered-dose inhalers (pMDIs). The chemical structure of HFO-1234ze(E) has the $-\text{CF}_3$ moiety, which is associated with formation of TFA after atmospheric degradation. In an earlier study, we coupled atmospheric GEOS-Chem modelling with fate-and-transport modelling to estimate TFA levels due to HFO-1234ze(E) emissions from prospective pMDI usage in the Rhine region, estimating TFA deposition fluxes between 0.9 and 3 $\mu\text{g}/\text{m}^2/\text{yr}$ [1]. Building on those deposition fluxes, we applied the methodology recommended in the US-EPA Human Health Risk Assessment Protocol (HHRAP) [2] to simulate chemical transport in the food chain using TFA-specific physicochemical inputs to predict TFA concentrations in edible plants and meats across Rhine sub-basins. Consistent with TFA's low hydrophobicity and limited sorption, modelled concentrations equilibrated rapidly (within months in all modelled environmental matrices). Steady-state concentrations were 85–150 ng/kg in total of above-ground and below-ground produce, 29–52 ng/kg in crop (wheat), and less than 0.34 ng/kg in meat (beef, pork, and chicken). These negligible TFA concentrations in foods provide the dietary inputs for subsequent Stochastic Human Exposure and Dose Simulation (SHEDS) modelling alongside inhalation, drinking water, and soil pathways. Our SHEDS modelling predicts that cumulative TFA intake rates for humans due to pMDI-based HFO-1234ze(E)-derived TFA in the environment are more than 150,000-fold below the existing threshold (30,000 ng/kg/day) of acceptable daily intake (ADI) for TFA established by European Food Safety Authority (EFSA).

Disclaimer/Disclosure:

SGT, PG, and HK are employees of AstraZeneca and hold shares/share options in AstraZeneca. KZ and KV are employees of Ramboll.

3.02.P-We181 Numerical modelling of water discharge dispersion from offshore hydrogen production : The Gulf of Lion case study

Nicolas Michelet, Matthieu Dussauze, Marie Robert and Lénaïg Mellaza, France Energies Marines, France

As part of its carbon neutrality strategy, the European Union has set ambitious targets for offshore wind energy deployment. However, this expansion alone will not be sufficient to meet the growing demand for clean and sustainable fuels required to reduce reliance on fossil resources. In this context, hydrogen (H₂) offers a promising alternative to conventional power export, and stakeholders in Offshore Wind Farms (OWFs) are increasingly considering the integration of H₂ production within OWF facilities. In offshore settings, H₂ production generates significant volumes of Produced Water (PW), defined as a mixture of brine from desalination systems used to supply freshwater for electrolysis and cooling water from electrolysis units. These waters exhibit elevated temperature and salinity compared to ambient conditions and contain chemical additives used during water treatment. Since it is generally accepted that those waters will be discharged directly into the marine environment, its management has become a growing concern. To assess the potential environmental impact of such discharges, a 990 MW wind farm was simulated in a microtidal marine environment using two numerical models: PLUME2.0 for local-scale dispersion and CROCO for regional-scale transport. At the local scale, near a wind turbine or substation, PW dispersion showed rapid dilution within a short distance, with temperature and salinity gradients falling below natural daily variability after only dozen meters. At the regional scale, in the Gulf of Lion, the distribution of maximum chemical concentrations was primarily driven by dominant hydrodynamic processes. Furthermore, in such environments, the use of worst-case scenarios based on maximized production appears unsuitable for environmental impact assessment and highlights the need for more realistic operational scenarios.

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3.02.P-We182 Numerical modelling of chemical dispersion from offshore hydrogen production

Nicolas Michelet, Matthieu Dussauze, Marie Robert and Lénaïg Mellaza, France Energies Marines, France

As part of its carbon neutrality strategy, the European Union has set ambitious targets for offshore wind energy deployment. However, this expansion alone will not be sufficient to meet the growing demand for clean and sustainable fuels required to reduce reliance on fossil resources. In this context, hydrogen (H₂) offers a promising alternative to conventional power export, and stakeholders in Offshore Wind Farms (OWFs) are increasingly considering the integration of H₂ production within OWF facilities. In offshore settings, H₂ production generates significant volumes of Produced Water (PW), defined as a mixture of brine from desalination systems used to supply freshwater for electrolysis and cooling water from electrolysis units. These waters contain chemical additives introduced during treatment, such as chlorine, which is widely employed as a biocide in desalination and cooling processes. It is commonly admitted that those waters will be discharged directly into the marine environment, making their management an issue of growing importance. To assess the risks associated with such discharges, a 990 MW wind farm was simulated over the future 'Centre Manche 1' site using the CROCO model to estimate chemical dispersion and the corresponding Predicted Environmental Concentration (PEC). In this tidal environment, the distribution of maximum concentrations was primarily driven by tidal currents. Additionally, due to the highly dynamic conditions that promote dilution, PEC values estimated under both maximized and realistic scenarios were comparable. This indicates that a simplified maximized scenario is sufficient to provide a consistent PEC estimation and support subsequent risk assessment.

3.02.P-We183 Environmental Risk Assessment of Product Type 18 Biocidal Products: A Comparative Analysis of Emission Scenario Document 2008 and 2024 Using Real-World Applications

Kishor Acharya, Eva Tallentire, Martin Hilton, James Pemberton and Alison McGuire, Exponent International Ltd., United Kingdom

Environmental Risk Assessment (ERA) for biocidal products relies on Emission Scenario Documents (ESDs) to estimate exposure. Product Type 18 (PT18), covering insecticides and acaricides and products to control other arthropods, has undergone a major update: the 2024 ESD for household and professional uses replaces the 2008 version and applies to active substance approvals from June 2025, with product authorisation requirements

effective December 2026.

The new ESD introduces refined emission scenarios for residential and non-residential buildings based on a new scale concept and treatment patterns with new default parameters. This includes a revised approach for the derivation of the simultaneity factor and the wet cleaning efficiency. New emission scenarios are introduced for example, for bed-bug treatments, aircraft disinfection and large-scale outdoor spraying and new chapters on risk mitigation measures (RMM) and seasonal control. The guidance also presents a newly developed quantitative primary poisoning assessment and a revised secondary poisoning assessment.

The full extent of these changes on the ERA outcomes remains unclear. The 2024 ESD introduces a fundamentally different framework from the 2008 version, so exposure estimates and risk characterisation may vary significantly. This could affect active substance and product renewals, requiring additional data or refined risk assessments. Until experience with the new approach accrues, the impact of the new guidance creates regulatory unpredictability for future submissions.

To help address this, a comparison of common indoor and outdoor PT18 uses has been performed, applying both the 2008 and 2024 ESD frameworks. Its findings will help inform compliance strategies and support stakeholders in adapting to the new requirements.

3.02.P-We184 Optimal Ecological-Economic Harvesting of Bioaccumulators for Aquatic Bioremediation
Sebastian Verslycke¹ and Michael Neubert², (1)United World College, United States, (2)Woods Hole Oceanographic Institution, United States

Aquatic ecosystems worldwide face persistent contamination from bioaccumulative pollutants, increasing the need for cost-effective and ecologically grounded bioremediation strategies. Fish populations capable of bioaccumulating environmental pollutants provide a potential pathway for pollutant removal through targeted harvesting. We developed a dynamical model that integrates fish population growth and contaminant uptake within an explicit economic framework capturing harvesting costs, environmental externalities, and revenue associated with biomass extraction. While both bioremediation and optimal harvesting have been examined in prior studies, their explicit integration within a single eco-toxicological and economic model remains uncommon. We used our model to evaluate potential management strategies by formulating an optimal control problem that maximizes net societal benefit by balancing economic returns from biomass removal against the costs of harvesting effort and environmental contamination. The estimated model conditions provide a set of economically and ecologically feasible harvesting trajectories that maintain long-term population viability while enhancing pollutant removal efficiency. These findings highlight parameter regimes under which targeted harvesting can substantially improve remediation outcomes relative to unmanaged or conventional strategies. Overall, our modeling approach offers a quantitative basis for evaluating how coupled ecological and economic processes can be leveraged to support cost-effective bioremediation programs in contaminated aquatic systems.

3.02.P-We185 Chemical Risk Assessment Using Control Banding in the Safe and Sustainable by Design Framework: Application to Fuel Cell and Electrolyser Manufacturing
Manon Lisiecki and Joséphine STECK, Univ. Grenoble Alpes, CEA LITEN DTNM, France

Control banding provides a practical and accessible approach to chemical risk assessment, especially when traditional predictive methods are overly complex, costly, or require detailed data that may not be available in early stages. The proposed methodology is aligned with the Safe and Sustainable by Design (SSbD) framework proposed by the European Commission, by addressing safety throughout the product lifecycle. It integrates hazard identification with exposure scenarios and prioritize safety measures via the STOP principle (Substitution, Technical, Organizational, Personal). Two crucial products toward safer and more sustainable hydrogen production with the same functional unit as previous sustainability assessment are evaluated: the manufacturing of a fuel cell (PEMFC) and an electrolyzer cell (SOEC). First, the chemicals involved are inventoried and their hazards assessed with a score based on the Material Safety Data Sheet recalculating mixture hazards via regulation thresholds using Quarks Safety® software. Then, the exposure scenarios combine fixed (quantity, frequency) and adaptable (process type, protection equipment) parameters. The Gross Risk is defined under worst-fixed exposure parameters (no ventilation, fully exposed) for each chemical. Then, residual risks are calculated for the multiple exposure scenarios thanks to adaptable parameters (worker protection, closed-process, source of ignition) and control levels. Due to the high number of chemicals involved, a categorization of gross risk (low to high) is proposed. For highest-risk chemicals (e.g., solvent, ceramic slurries), substitution with less hazardous alternatives is recommended. Medium-risk require combined technical

and organizational controls, while low-risk solely require personal measures. This approach enables comprehensive risk reduction, identifying hazardous chemicals, addressing data gaps in product development while ensuring compliance with EU safety regulations. All the results are summarized into a self-assessment guide for rapid, time-efficient chemical risk evaluation in hydrogen product manufacturing. Unlike current SSbD safety approaches, it stands out by rapidly delivering semi-quantitative results using available data. By identifying inherently safer alternatives, and by optimizing processes that simultaneously improve worker protection and resource efficiency, it demonstrates that safety and sustainability can be complementary rather than competing priorities.

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Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the Clean Hydrogen Partnership. Neither the European Union nor the granting authority can be held responsible for them. The project is supported by the Clean Hydrogen Partnership and its members.

3.02.P-We186 Evaluating Metabolism and Accumulation of Polycyclic Aromatic Hydrocarbons in Juvenile Hybrid Striped Bass in Support of PBTK Modeling Efforts

Gregory Martin Langlois and Peter van den Hurk, Clemson University, United States

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in marine/aquatic waters and sediments, and can have a range of toxic effects on aquatic organisms. This project aims to develop a physiologically based toxicokinetic (PBTK) model for the uptake of aqueous pyrene in juvenile hybrid striped bass, to model the absorption, distribution, metabolism and excretion of this compound. At this moment there are no established PBTK models for hybrid striped bass, despite the growing popularity of this animal as a sport fish and food source in North American waters. In the pursuit of developing this physiologically based toxicokinetic model, it is essential to define and parameterize both the physiological components of the model organism as well as the physiochemical properties of the compound selected for modeling.

One of the key components in PBTK models is biotransformation. In an effort to generate metabolic elimination parameters for a juvenile hybrid striped bass PBTK model, S9 liver fractions are used to perform overall elimination assays for a range of PAHs, including pyrene, which is the primary focus of this work, as well as a panel of other applicable PAHs to provide elimination rate constants for use in modeling efforts. Additionally, a characterization of the metabolic activity of juvenile hybrid striped bass for specific enzymes involved in PAH metabolism, such as CYP1A1 using the EROD assay, as well as UDP-glucuronosyltransferase activities, were performed to better understand the elimination pathways of PAHs in this poorly characterized species.

Additionally, to calibrate the PBTK model, free swimming exposures to aqueous pyrene were performed to calibrate model predictions to real time chemical exposures to evaluate the predictive capabilities of the developed PBTK model.

Keywords: Physiologically Based Toxicokinetic Modelling, ADME, Metabolism

3.02.P-We187 Advancing Geo-Environmental Risk Assessment for Residual Mercury in Soil: Modeling Mercury transformations and Exposure Dynamics

Monami Kondo, Yasuhide Sakamoto and Junko Hara, National Institute of Advanced Industrial Science and Technology, Japan

Mercury (Hg) is a globally circulating substance that moves between atmospheric, terrestrial and aquatic reservoirs through complex biogeochemical processes. While exposure and risk management through various Hg regulations have advanced, substantial quantities of Hg continue to enter the environment from localized emissions originating in industrial zones and mining areas. Particularly at artisanal and small-scale gold mining (ASGM) sites, Hg exposure may occur without awareness of its hazards, necessitating the promotion of worker safety education and transition to alternative methods. Therefore, it is essential to visualize Hg exposure risks and encourage restrictions on access to hazardous sites and groundwater use. To address this necessity, we aim to develop a model for assessing the short- and long-term exposure risks of residual Hg discharged at ASGM sites. We have already developed a geo-environmental risk assessment system (GERAS-3) and also enhanced it to enable the representation of time-variant atmospheric transport of volatile compounds such as Hg and volatile organic substances (VOCs) in unsaturated soil. Furthermore, this model can reproduce changes in mass transfer behaviour in response to meteorological conditions such as rainfall and air pressure changes at the soil-atmosphere boundary. We are further refining this model to incorporate form changes and chemical species in

both anaerobic and aerobic soil environments, enabling it to account for various Hg forms. This would enable the model to provide a more detailed analysis of Hg immobilisation within the soil and its release into the atmosphere and groundwater. In the presentation, we will discuss the advantages of understanding environmental behaviour and spatial distribution of Hg, including its morphological changes, for establishing a methodology to assess Hg inhalation exposure under specific scenarios.

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3.02.P-We188 Inverse TOXSWA Modelling for Level P-II Water-Sediment Kinetics: A Robust Approach *Gerald Reinken¹, Klaus Hammel¹, Kristina Hoffmann² and Ruth Porschewski², (1)Bayer AG, Research & Development, Crop Science, Germany, (2)knoell Germany GmbH, Germany*

Water-sediment degradation studies are currently evaluated according to FOCUS kinetics guidance at Tier-1 (P-I) and Tier-2 (P-II) levels. While Tier P-I relies on total-system half-lives, Tier P-II requires separate kinetic fits for water and sediment compartments including exchange processes. Due to strong parameter correlations and identifiable issues, these kinetic evaluations can give inconclusive results and force the use of regulatory default worst-case values (e.g. $DT_{50} = 1000$ d) in subsequent exposure modelling. In such cases, higher-tier kinetic evaluations by inverse TOXSWA are a regulatory recognised option as described in the FOCUS kinetics guidance from 2014.

This work demonstrates the successful application of inverse modelling with TOXSWA version 1.2 at Tier P-II for one parent substance across four laboratory water-sediment systems. The approach provided well-identified and robust kinetic endpoints: $DT_{50, \text{water}} = 98\text{--}117$ d, $DT_{50, \text{sed}} = 88\text{--}336$ d, and mass-transfer coefficients $k_{\text{ex}} = 0.14\text{--}0.22$ m³/kg. All three key parameters (DT_{50} water, DT_{50} sediment, and k_{ex}) were reliably and simultaneously fitted for every system with narrow confidence intervals.

These results demonstrate that inverse modelling with TOXSWA is a powerful, scientifically robust, and defensible higher-tier refinement tool at Tier P-II. It is therefore recommended that this approach be explicitly recognised and retained as an acceptable option in future revisions of the EFSA/FOCUS kinetics guidance.

3.02.P-We189 Revisiting MACRO 5.2 Parameterization for FOCUS Surface Water Scenarios *Gerald Reinken, Bayer AG, Research & Development, Crop Science, Germany*

The MACRO model is a cornerstone of pesticide fate assessment within the FOCUS Surface Water (SW) framework, providing a physically based simulation of macropore flow and solute transport in structured soils. The original FOCUS SW drainflow scenarios were parameterized using MACRO 4.3b, calibrated and validated against field data to ensure realistic hydrological behaviour.

With the release of MACRO 5.2, several technical updates were implemented, including changes in numerical routines, parameter management, and batch-execution functionality. While these updates improve model flexibility and performance, early comparisons indicated that outputs for standard FOCUS SW scenarios may deviate from those generated with the earlier version, potentially affecting regulatory consistency.

This work outlines a systematic approach to evaluate and better harmonize MACRO 5.2 with well established FOCUS drainflow benchmarks. A detailed comparison of process implementation and parameterization between MACRO versions 4.3b and 5.2 identified three categories of changes:

- 1) Modified parameter input values (e.g. numerical layers increased from 15 to 60; scenario-specific FRACMAC and replacement pre-defined PET values)
- 2) Newly introduced parameter and processes (e.g. internal EVAPORATE calculation; full dual-permeability formulation requiring explicit domain-exchange coefficients and aggregate half-width; optional scaling for OC partitioning in macropores)
- 3) Adjusted numerical solver technique (still using an implicit finite-difference method, but with a more precise numerical approximation for episodic macropore flow)

A structured global sensitivity analysis has been initiated using MACRO 5.2's Monte Carlo and batch-run

modules across all official FOCUS SW drainflow scenarios (D1–D6). Key parameters governing macropore flow are identified, and adjustment ranges are tested to restore better agreement with 4.3b reference loads while maintaining physical plausibility. The results are intended to serve as a basis for updated guidance on the regulatory use of MACRO 5.2 in European pesticide risk assessment.

3.02.P-We190 Spatial Representativeness and Temporal Variability in the FOCUS Surface Water Framework: Implications for Future Endpoint Selection

David Patterson¹, Beate Erzgraeber², Neil Mackay³, Monika Maleri⁴, Peter Rainbird⁵, Gerald Reinken⁶, Dieter Schaefer⁶ and Gregor Spickermann⁷, (1)Syngenta Ltd, United Kingdom, (2)BASF, Germany, (3)FMC Corporation, United Kingdom, (4)Corteva Agriscience, Germany, (5)Syngenta Ltd, Switzerland, (6)Bayer Research & Development Services LLC, Germany, (7)ADAMA, Germany

The FOCUS Surface Water (SW) framework remains the central EU approach for evaluating aquatic exposure to plant protection products. The 2020 FOCUS SW “repair action” advanced temporal representativeness by extending all scenarios to a 20-year assessment period. However, current discussions regarding the regulatory interpretation of aquatic exposure predictions resulting from FOCUS SW Repair risk overlooking the equally foundational spatial basis of protectiveness embedded in the original scenario design. The FOCUS scenarios were explicitly constructed from agro-environmental combinations of climate, soil texture, slope, drainage prevalence, land use and cropping, parent material and hydrological connectivity in a catchment to represent realistic “worst-case” conditions for drift, runoff and drainage. Their representativeness is a function of both spatial and temporal components.

We re-evaluate the spatial coverage and consider the percentile “worst-caseness” of the FOCUS scenarios using a 2007 extended spatial analysis for the EU25, which quantified the national extent of each scenario’s agro-environmental envelope. This analysis confirms that not all scenarios represent the same spatial percentile: some encompass extensive fractions of EU agricultural land, while others represent high-end, spatially scarce combinations of slope, drainage density or soil erodibility. This heterogeneity of spatial coverage and “worst-caseness” needs to be considered in conjunction with temporal variability. Certainly there may be lessons to be taken from the FOCUS groundwater approach in which an overall 80th percentile is achieved through the combination of spatial and temporal 90th percentiles. The additional complexity for surface water assessments concerning rules regarding application timing and drift loading percentiles should also be considered.

By combining the 2007 EU25 spatial quantification with the 20-year temporal outputs introduced in the repair action, we highlight how spatial and temporal considerations interact to yield the current protective benchmark. We argue that selection of regulatory endpoints from the 20-year simulation period must continue to reflect this dual foundation. Ignoring the spatial percentile risks shifting the balance between representativeness and conservatism that underpinned the original FOCUS surface water guidance.

3.02.P-We191 Field-Scale Run-off Vulnerability Mapping Using GIS Modelling to Support Precision Agriculture

Olha Khomenko¹ and Mercedes Franey-Gardiner², (1)Cambridge Environmental Assessments (CEA), Helsby, United Kingdom, (2)Cambridge Environmental Assessments (CEA), United Kingdom

Diffuse agricultural pollution from field run-off contributes significantly to water quality degradation, yet conventional farm management typically applies uniform practices across fields despite substantial spatial variability in run-off risk. This study aims to demonstrate how a GIS-based modelling can allow a field-specific run-off vulnerability mapping to enable targeted precision agriculture interventions. The methodology integrates multiple field-specific spatial data layers, including high-resolution digital elevation models for slope analysis, local hydrology networks, soil properties, and proximity to water courses.

Using weighted overlay analysis and hydrological modelling tools, we identify areas within individual fields where run-off of nutrients, pesticides, and sediments is most likely to occur. To enhance interpretability and support decision-making, we are developing a complementary machine learning component using random forests to classify field zones as high, moderate, or low run-off risk based on the GIS-derived variables. While the localised geology means models trained on one catchment may not transfer directly to others, the methodology itself is broadly applicable and can be calibrated for new regions with locally available data. Combining classical machine learning techniques with GIS approaches provides a deterministic, process-informed index of run-off susceptibility, evaluates and optimises variable importance, and classifies risk zones, improving predictive accuracy and interpretability. Although demonstrated in a single catchment, the modelling framework is designed for calibration and deployment in diverse agricultural landscapes.

While the existing run-off risk tools typically operate at field or catchment scale, our model aims to resolve risk at sub-field resolution, enabling targeted interventions. This precision approach has the potential to reduce agricultural chemical loading to water bodies while maintaining crop productivity, offering economic benefits to farmers through reduced input costs and environmental benefits.

3.02.P-We192 Outdated Forecasts: Climate Drift and the Future of Pesticide Risk Models

Olha Khomenko¹ and Mercedes Franey-Gardiner², (1)Cambridge Environmental Assessments (CEA), Helsby, United Kingdom, (2)Cambridge Environmental Assessments (CEA), United Kingdom

Regulatory risk assessments for Plant Protection Products (PPPs) in Europe rely on FOCUS scenarios, which use historical weather data. However, the climate has shifted since this baseline period, bringing changes to the crop calendars, precipitation patterns and average temperatures, raising questions about whether the current models' parameterisation is fit for purpose in 2025 and beyond.

The frequency and intensity of heavy rainfall events have increased significantly over the past decade, with more frequent extreme events. Temperatures across the UK have increased since the 1961-1990 baseline period, with recent years (2022-2024) averaging 9.8-10.0°C compared to the historical baseline of 8.3°C. While these changes may appear moderate, it remains unclear what level of change they bring to the model results. Using outdated values can potentially alter pesticide fate predictions as the higher temperatures increase degradation and volatilisation rates, while more frequent intense rainfall can lead to a higher surface run-off and leaching to groundwater, with seasonal shifts toward wetter winters and drier summers changing peak exposure timing. This study aims to investigate how rapidly climate change is rendering FOCUS weather files obsolete and aims to determine the frequency at which parameterisation datasets require updating to maintain regulatory relevance. We analyse how temperature and precipitation change in the Okehampton FOCUS groundwater scenario, comparing historical baseline conditions with observed climate data from 2000-2024. Using pesticide fate models, we quantify how these climatic shifts affect predictions of pesticide leaching and run-off. Key deliverables will include precipitation and temperature shift thresholds at which climate drift compromises model reliability, evidence-based recommendations for update frequency. This research will provide regulators with the evidence base needed to modernise pesticide risk assessment approaches and ensure protective standards remain robust in an era of accelerating climate change.

3.02.P-We193 Screening of PFAS Dynamics in the Rhine and Meuse Catchment: Future Scenarios in SimpleBox

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Minimizing exposure to per- and polyfluoroalkyl substances (PFAS), for example via food and drinking water, is crucial to protect human health. In the Netherlands, approximately 40% of drinking water is sourced from surface water, mainly from the Rhine and Meuse rivers. The presence of PFAS in these rivers is therefore a concern, especially given the limited possibilities to effectively remove these substances during water treatment. This study aims to assess the future PFAS burden in surface water as Dutch drinking water source. The central research question is: How long will PFAS remain in the Rhine and Meuse after the implementation of emission-reducing measures? This was analyzed using a screening level multimedia fate model (SimpleBox) for conducting a semi-dynamic analysis of PFAS fate and transport in time. The landscape scenario was adapted to reflect the specific characteristics of the Rhine and Meuse River basins, including precipitation variability. Seven PFAS were selected based on their high prevalence in Dutch drinking water samples derived from surface water: PFBS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, and TFA. For each of these substances, physicochemical properties and degradation rates were obtained. Several time-explicit emission scenarios were analyzed. Preliminary scenarios include a stringent scenario, involving complete emission reduction within a 10 to 15-year timeframe, and less stringent scenarios, such as a 5% derogation and the implementation of additional transition periods.

The preliminary results show that for the most stringent scenario a 99.9% reduction in PFAS in Rhine and Meuse water would be reached within 15 to 25 years. This is without consideration of groundwater as additional source. In less stringent scenario's the maximum level of reduction varies together with the time span to reach them. Differences between the PFAS compounds are relatively small, except for TFA and PFOA. The reduction level relevant for drinking water production depends on both the current concentrations of PFAS and the relative potency of the individual PFAS compounds. This was further analyzed using the Relative Potency Factor (RPF) approach in combination with recent measurements of PFAS in Rhine and Meuse.

This study offers insights into the purification measures that may be required for the Rhine and Meuse rivers in the future, and identifies key areas for further research.

3.02.P-We194 Climate-Driven PFAS Risk Prediction: Incorporating Rainfall Dynamics into Contamination Forecasting

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Per- and polyfluoroalkyl substances (PFAS) contamination of water sources used for drinking water is a growing public health concern. However, the influence of extreme weather events on PFAS transport and concentration dynamics remains poorly understood.

This study develops a predictive framework for PFAS contamination that moves beyond conventional spatial-only models. Specifically, we model PFAS mobilisation triggered by extreme rainfall events, integrating precipitation intensity as a dynamic driver of contamination risk. The framework links climate extremes directly to risk forecasting, offering a climate-informed tool for safeguarding drinking water supplies under intensifying weather variability.

We use public monitoring data to link the measured PFAS concentrations in UK drinking water sources with spatial and environmental predictor variables, including land use patterns, soil characteristics and precipitation intensity. Multiple classical ML algorithms, including random forests, gradient boosting machines, and support vector machines, will be trained to identify conditions under which PFAS concentrations spike in source waters. The impact of increased precipitation specifically examines whether extreme events have the potential to trigger a significant increase in PFAS concentrations, and whether these mobilisation events can be predicted sufficiently in advance to enable drinking water providers to implement mitigating strategies or alternative source water strategies.

This approach will enable utility providers to proactively respond to PFAS contamination threats during extreme weather, thereby improving protection of drinking water supplies and public health outcomes in an era of intensifying climate extremes. The novelty of this work lies in its explicit treatment of rainfall-driven contamination spikes, which are largely overlooked in existing PFAS machine learning models that focus primarily on spatial distribution patterns.

3.02.P-We195 Stay updated: use of Bayesian learning algorithms for updating a probabilistic exposure model with chemical monitoring data

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A suite of process-based models are available for predicting the environmental release, transport, fate and exposure of chemical substances and mixtures in the aquatic environment. However, even advanced models do not always make optimal use of the latest scientific techniques or relevant available datasets. Precautionary principles are often incorporated in models to account for lack of information or other forms of uncertainty. Here, we present a large-scale study where Bayesian techniques are being explored for efficient integration of process-based model predictions with observations from river monitoring sites. Our starting point is the European-scale process-based fate model from the former EU project SOLUTIONS. Within the project ENCORE (ENVIRONMENTAL CO-exposure and Risk Estimation), this model has been revised and expanded with stochastic simulations (hereafter referred to as the ENCORE fate model). To further leverage the ENCORE fate model simulations, we will make use of river monitoring data from the European Environment Agency's Water Information System for Europe (Waterbase - Water quality), which contains observations of hazardous substances from more than 50 million samples across Europe. To this end, we have developed an object-oriented Bayesian network (BN) model representing the main properties and outputs of the ENCORE fate model, expressed as discrete probability distributions. This OOBN allows us to explore the use of Bayesian updating techniques at different levels of spatial aggregation, for different purposes such as the following:

- (1) River Basin District level - BN model exploration: evaluation of approach.
- (2) Water body level - refined BN model predictions: Bayesian inference for improved accuracy of exposure characterisation at higher spatial resolution.
- (3) Monitoring site level - parameter learning: Bayesian parameter learning for refined BN model settings (conditional probability tables).

This poster will present results from a pilot study with selected pesticides in Belgium. Other aspects of the ENCORE project will be covered by other presentation planned for this meeting: Work flow, information sources, data processing; the ENCORE fate model; and probabilistic approaches to mixture risk calculation.

Disclaimer/Disclosure:

The ENCORE project is sponsored by the European Chemical Industry Council (CEFIC) as part of the Long Range Initiative (LRI) under project ECO-66 “Next generation risk assessment methods supporting the identification of environmental co-exposures of potential concern”.

3.02.P-We196 Modelling the Fate of Chemicals from Untreated Wastewater Discharges: A Routed and Spatially refined Model

Simran Sandhu¹, Juliet Hodges², Hugh Barlow¹ and Stephen Glavin¹, (1)Unilever, United Kingdom, (2)Safety & Environmental Assurance Centre, Unilever, United Kingdom

Untreated Wastewater Discharge (UTWWD) poses a significant risk to river ecosystems globally, particularly where populations lack connection to Sewage Treatment Plants (STPs). Such discharges introduce high loading of sanitary determinands (notably unionised ammonia), as well as suspended solids, and down-the-drain chemicals directly into freshwater systems. Elevated ammonia and chemical concentrations can lead to oxygen depletion, toxicity, and disruption of aquatic communities, threatening biodiversity and ecosystem function. We present a routed and highly spatially refined model that determines both chemical loads and ammonia concentrations across entire river networks. The model integrates high-resolution population connectivity data, geospatial STP and other disposal mechanism infrastructure, and the HydroATLAS hydrological database to allocate unconnected populations to their nearest river segments. For each segment, chemical and ammonia loads are estimated based on population discharge, water use, and chemical-specific parameters (e.g., half-life). The model simulates routed transport and decay, identifying “impact zones” where ammonia concentrations exceed ecological thresholds and differentiates chemical decay based on the different water conditions. Outside these impact zones we map the concentrations, distribution and potential risks of down-the-drain chemicals. Importantly, outside the impact zone, concentrations of down-the-drain chemicals are predicted to be lower than within the impact zone. In the poster we will present a case study with preliminary results from Beijing’s river network, showing distributions of rivers within the impact zone and mapping concentrations of down-the-drain chemicals throughout rivers along with their potential impacts. Future development will focus on integrating STP effluent to assess the combined impacts of treated and untreated wastewater and validating predictions with monitoring data. This spatially refined and routed approach provides a robust foundation to scale to global river networks and provides a higher-tier model to supporting next generation risk assessment (NGRA). These advances enable science-based decision-making for regulators, industry, and water managers.

3.02.P-We197 Generating a Robust Global Dilution Factor Estimation from River Flow and Wastewater Effluent

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We present a global, high-resolution dataset of dilution factors (DFs) for chemical risk assessment in rivers, improving spatial and temporal resolution compared to existing datasets. DFs are critical for estimating environmental concentrations of contaminants discharged from wastewater treatment plants (WWTPs) into river systems. Currently, DFs used in chemical risk assessments are limited by spatial resolution, temporal depth, and methodology transparency. DFs individual river segments tend to vary according to natural variability in stream flow over time, and spatially across a landscape underscoring how the utility of a single default screening level DF is limited for refined environmental risk assessments. This work addresses these gaps by developing an up-to-date global DF dataset using high-temporal resolution hydro-environmental data and robust geospatial processing.

The dataset integrates HydroSHEDS river networks, HydroWASTE WWTP locations, and FLO1K flow grids representing annual mean and lowest monthly flows for 56 years. All FLO1K grid cells overlapping each river segment were analysed and a representative flow point (i.e., grid cell) selected.

DFs were computed as the ratio of river flow to WWTP discharge and summarised at multiple spatial scales (country, basin, administrative units) and temporal dimensions (annual, 30-year averages, and percentiles):

- 1: Raw STP-linked flow and discharge data annually.
- 2: Processed STP-level DFs with temporal percentiles (5th–95th).
- 3: Aggregated summaries by country and basin, including filtered datasets for rivers >0.1 cubic meters per second flow (best practice).

Global results reveal extreme variability in dilution potential: median DFs range from <10 in arid regions to >10⁷ in high-flow systems (for rivers exceeding 0.1 cms flow). Results also revealed considerable temporal variability over a 56-year window for individual rivers, highlighting the value of utilizing a range of flow values for DF generation. This work emphasizes the importance of spatially and temporally explicit DF estimates for

chemical risk modelling. The dataset supports probabilistic exposure assessments informing regulatory frameworks by providing transparent, reproducible metrics. By presenting both methodology and data, this novel work establishes a benchmark resource for environmental safety evaluations, enabling integration into contaminant fate models and supporting global initiatives on water quality and chemical risk.

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3.02.P-We198 Monitoring and Modelling Exposure to 6-PPD Quinone in UK Rivers

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6-PPD is a chemical antioxidant used in tyres to prevent degradation and extend the tyre's lifespan. Following release, it reacts with ozone to form 6-PPD quinone (6-PPDq) which can then be transported to surface water. The presence of 6-PPDq in highway runoff in the North-West of the United States has been linked to impacts on Coho salmon. While the UK, is a heavily populated country with areas of high traffic density, the occurrence and risks of 6-PPDq in the country have yet to be established. The aim of this study was to develop and evaluate a high temporal and spatial exposure assessment model for 6-PPDq and other highway-derived pollutants in UK surface waters. The model, which was developed for the Yorkshire region in England, utilised information on traffic densities, 6-PPD release rates, rainfall alongside a hydrological model and a river model to estimate concentrations of 6-PPDq in 16,000 sub-basins across Yorkshire. Alongside the modelling, continuous monitoring of surface water was performed at 19 locations across the region to generate weekly data on the occurrence of 6-PPDq to provide a dataset to evaluate the model. In the monitoring study, 6-PPDq was detected in 27% of the samples obtained and in between 70-80% of samples from the more urbanised monitoring locations. Maximum measured concentrations were seen in the more urbanised areas and were in the low ng/l range. Comparison of model predictions with the monitoring results showed model predictions to be fair and ranging from 1.3 to 6.6 times higher than the observed concentrations. Use of the model for the 16,000 sub-basins showed that more than 95% of sub-basins had concentrations lower than 5 ng/l but there were some situations where levels were high. The sub-basin with the highest exposure concentration had an annual average concentration of 72 ng/l and a peak concentration of 3500 ng/l. Our modelling approach provides a useful approach for characterising exposure and risks of 6-PPDq and other highway-derived contaminants and for identifying hotspots of exposure.

3.02.P-We199 Now You See It, Now You Don't: Describing Patterns in Environmental Chemicals at the Edge of Detection

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Since the 19th century, analytical chemists have known about the concepts of limits of detection (LoD) and minimum recorded value (MRV). To summarise environmental chemistry data containing 'less-thans', we can use statistical techniques that inherit from epidemiological and actuarial science. Yet we want to move beyond data summaries to get the most from messy real-world monitoring data that vary in frequency across multiple sites: patchy datasets with a potentially high proportion of observed 'less-than' concentrations. We want to derive trends in chemical exposure for comparison with environmental standards. And at broad scales we want to gauge the success of policy and warn of emerging risks. We must avoid artifacts arising from changes in LoD/MRV as analytical methods have improved and would prefer to calculate confidence intervals for our estimates. Fortunately all this can be achieved within the broad statistical framework of generalised additive mixed modelling.

We present an approach for the analysis of trends in left-censored (containing less-than) chemical concentration time series from multiple river water monitoring sites with data gaps and varying sampling frequencies. We:

1. Incorporate spatial structure;
2. Describe observed concentration data not by a single number but as two columns indicating upper and lower boundaries. Behind the scenes, less-than values are treated as cumulative rather than point density estimates of probability.
3. Let the data describe potential non-linearities in an efficient manner;
4. Decompose the trend into annual and seasonal components;

5. Manage temporal correlation, but with monthly (or less) frequencies this is often not an issue;
6. Produce trends across multiple spatial scales from national down;
7. Derive optimal site-level predictions, even for sites with minimal data;
8. Optionally attempt to explain spatial and temporal patterns using sample and site-level covariates.

Using data for dissolved metals and trace organic chemicals, we illustrate how trends can emerge incrementally from the 'mess' of the raw data. We illustrate how, when analysed incorrectly, such trends can be biased, overly-certain or just missed. Implementing such approaches programmatically allows us to automate, reproduce and re-use the data extraction, analysis and graphical presentation. In England, while trends in metals and some organic chemicals are clearly downwards, this is not universally the case.

3.02.P-We200 Assessing the Significance of Heteroaggregation in Environmental Fate as a Tool for Nanoparticle Grouping

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Engineered nanoparticles (ENPs) introduced into aquatic environments are simultaneously subject to multiple removal processes, such as dissolution, sedimentation, advection, and aggregation. One of the main challenges in assessing the environmental fate of nanomaterials is accounting for aggregation processes.

In this study, we examined for which nanoforms heteroaggregation constitutes a sufficiently dominant process to shape their environmental behaviour. The analysis covered the aggregation propensity of 36 inorganic types of ENPs, each considered across multiple combinations of particle size and zeta potential. Using DLVO theory, attachment efficiencies (α) were estimated over a wide range of environmental conditions and subsequently incorporated into an environmental fate model. This enabled a direct comparison of aggregation rates with the kinetics of competing processes.

The results show that the likelihood of aggregation dominance varies widely — from a few to 100% — and depends on material composition, particle size, and zeta potential. Aggregation is nearly certain for metals and for small nanoparticles with near-neutral zeta potential, while its significance decreases with increasingly negative surface charge and varies depending on the bonding between atoms.

The analyses led to the development of a five-tier classification scheme that groups nanoparticles according to their expected environmental behaviour. The scheme enables rapid distinction between colloidally stable materials and those prone to aggregation. This tool aligns with current regulatory needs by providing criteria that support grouping and prioritisation of nanomaterials in environmental risk assessment.

Disclaimer/Disclosure:

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3.02.P-We201 Comparative Assessments of Environmental Plastics by Exposure Metrics in UTOPIA Modeling Scenarios

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Plastics are persistent environmental pollutants of increasing concern due to their extensive use and inadequate waste management. Once released into the environment, weathering processes promote their fragmentation into macro-, micro-, and nano-scale particles, which can interact with organic matter and microorganisms to form aggregates. Plastic fragments smaller than 100 μm might also biomagnify in coastal marine food webs, posing potential health risks to organisms. The environmental fate and transport of these plastics vary widely due to differences in particle shape, size, and density, and a wide variety of processes determine the environmental fate and lifetimes of plastic. Sustainability indicators have been proposed to guide plastic eco-design in terms of product lifetime or resource costs, however, metrics are also needed to assess environmental exposure to plastics. To address this gap, this study evaluates several possible exposure metrics for plastic pollution, including persistence and residence time, emission fractions, and committed dose to evaluate aspects of environmental exposure including reversibility and long-range transport potential. These metrics have been integrated into the UTOPIA unit-world model, which simulates the fate and transport of plastics with diverse physical properties, and can be used to comparatively assess environmental exposure to different plastics. Global sensitivity analysis with Monte Carlo simulations is applied to identify which particle characteristics most strongly influence exposure potential according to each metric. The results provide a quantitative modeling

perspective for the eco-design of next-generation plastics, offering insights into how physical properties can be optimized to reduce environmental persistence and exposure.

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3.02.P-We202 The Emission and Spread of Tyre Wear Particles

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Tire wear is the main source of microplastics in the Netherlands; most is released directly into the environment from traffic. Tire wear particles (TWP) consist of natural and synthetic rubbers and additives that may be harmful. When TWP reach vulnerable sites such as agricultural land or playgrounds, they can pose health risks. Sweco developed a spatial model using a Multi-Criteria Analysis (MCA) to map risks and identify hotspots. This study refines that model by estimating concentrations instead of distance-based risk factors and by applying transport equations to make dispersion visible. This allows exposures at vulnerable sites to be determined more precisely and helps policymakers set priorities.

Methods

Emissions per m² of road were calculated with an FME workflow that links BGT road segments to speed limits and vehicle?kilometers by road and vehicle type. Additionally, emissions per vehicle?kilometer were based on three road categories: motorway, non?urban, and urban. This resulted in a map of different emissions per road type. The emission map was combined with layers of vulnerable sites.

For transport simulation we are developing an FME/Python model that couples the Shallow Water Equations (SWE) to mass?transport equations, discretized with the Finite Difference Method (FDM). This approach has been applied to microplastics and other contaminants, but not previously specifically to in situ TWP. Roads and their immediate surroundings were classified by land cover. Because TWP dispersal typically remains within about 30 m, the computational domain is limited to reduce runtime.

Results and discussion

Motorways emit far more TWP: roughly 20× more than urban roads and 6× more than non?urban roads. However, high road density in built-up urban areas can cause accumulation, producing locally high concentrations and exposing vulnerable locations such as schools. The next step is time?dependent transport modelling; initial results are expected by the end of December.

Conclusion

Shifting to concentration estimates and transport modelling makes exposures more concrete. Accumulation in densely built urban areas with high road density can lead to unexpectedly high TWP concentrations, which supports targeted mitigation and prevention.

3.02.P-We203 Comparison of exposure modelling tools used in different regulatory areas

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In the frame of different regulatory areas of chemical substances (e.g. REACH, plant protection products, biocides) modelling tools are used for performing environmental risk assessment of these substances, requested under the respective regulation. The modelling tools predict in a first step the release of chemical substances into different environmental compartments after their use or during their service life. In a second step the modelling tools take into account the fate and distribution of chemical substances after release to different environmental compartments and predict an environmental concentration in different compartments. Depending on the regulatory area, the focus is on different environmental compartments and different release pathways.

We will give an overview on currently available standard tools and their use, also for the assessment of specific environmental compartments (e.g. FOCUS tools to simulate the release to groundwater or SimpleTreat to model a sewage treatment plant).

We further will show the limitations of such models for specific substances groups and will show recent and current developments of new IT tools. This includes also tools which attempt to cover different regulatory areas (e.g. Chesar platform for REACH and biocides). We will compare the models as far as possible in regard to

their input parameters, background distribution models and their output. One additional aspect is the flexibility of such models to replace default values with monitoring data in order to increase the realism of these tools and their predictions.

3.03 Advancing Environmental Monitoring through Ion Mobility Spectrometry coupled to HRMS: Innovative Strategies and Applications

3.03.T-01 Ion-mobility derived CCS-m/z trendlines for environmental contaminant screening – where are we and where can we go?

Lidia Belova, Maosen Zhao, Maarten Roggeman, Giulia Poma, Celine Gys, Paulien Cleys, Fatima den Ouden, Adrian Covaci and Alexander van Nuijs, University of Antwerp, Belgium

Ion mobility high-resolution mass spectrometry (IM-HRMS) derived collision cross section (CCS) values are a valuable additional identification parameter in suspect and non-target screening analysis (SSA/NTS) of legacy and emerging environmental contaminants. The utilization of CCS data is of special interest for contaminant classes occurring in homologue series as these show class-specific CCS-m/z trendlines. While the use of these trendlines for compound annotation has been documented in the context of per- and polyfluoroalkyl substances (PFAS), their applicability for the annotation of other classes of environmental contaminants remains largely unexplored.

The presented study focused on an extensive SSA study of >80 indoor dust samples collected at different locations (private housing, kindergartens, public buildings) in Belgium. After extraction and analysis by liquid chromatography drift-tube IM-HRMS, the obtained data were matched with a suspect list containing > 4000 compounds, covering various classes of indoor contaminants. Phthalates remained one of the major annotated classes showing the highest detection frequencies and semi-quantified concentrations. Alongside legacy phthalates identified at confidence level (CL) 1, annotated suspect phthalates covered homologue series with side chains ranging between C1 (dimethyl phthalate) and C12 (didodecyl phthalate). Additionally, homologue series of benzyl alkyl phthalates with varying side chains were annotated. Nevertheless, annotations were hampered by the occurrence of several isomeric structures for each homologue, resulting from differently branched side chains, allowing only an CL 3 annotation. Therefore, DTCCSN2 values were obtained for all analytes and were used to describe the corresponding CCS-m/z trendlines. For all suspect CECs, the experimental CCS values fell within the 95th percent confidence interval calculated for the CCS-m/z trendline deriving from available reference standards, contributing significantly to the confidence of the compound annotation. The reproducibility of observed trendlines was assessed between sample batches collected at different locations. The data is currently being evaluated aiming to characterize CCS-m/z trendline of additional emerging contaminant classes. On the basis of these examples, a discussion on an appropriate communication of the added identification confidence is conducted.

3.03.T-02 Holistic analysis of emerging contaminants in northern Antarctic Peninsula region waters through Liquid Chromatography–Ion Mobility Spectrometry–High-Resolution Mass Spectrometry

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The Antarctic Peninsula region is among the most remote and least populated regions on Earth. Despite the protective measures to safeguard its fragile ecosystem and geographic isolation, anthropogenic pressures are increasing. Early research provided evidence on the presence of persistent organic pollutants, capable of long-range environmental transport in such a remote area. In recent years, the occurrence of contaminants of emerging concern (CECs) has also been flagged and linked to human activity taking place in the region. These findings are based on targeted studies examining a limited range of compounds, leaving a significant gap in the comprehensive characterization of water chemistry pollution. To address this issue, advanced analytical techniques based on wide-scope target screening and non-target analysis (NTA) by means of liquid chromatography coupled with hybrid ion mobility spectrometry - high resolution mass spectrometry were applied to the analysis of 19 water samples collected from ice-free areas of Deception, King George, and Livingston Islands (South Shetlands archipelago). These methods support retrospective analysis, providing a broad-spectrum detection of compounds not previously identified or anticipated. Incorporating ion mobility adds a complementary dimension to compound identification, enhancing confidence in NTA results. A semi-

quantitative approach has also been employed to assess environmental risk. In total, 88 compounds were detected in at least one sample with different identification confidence levels: 53 of them were confirmed with reference standards (Level 1), 28 matched with spectral libraries (Level 2A), and 7 were annotated with Level 3, following the Celma et al. (2020) criteria. Ion mobility data enabled the identification of conformational isomers, such as adenosine, and differentiation of isobaric compounds like paraxanthine and theobromine. Most compounds presented concentrations in the ng/L range (91.8%), although a few of them reached the µg/L level. Industrial chemicals, personal care products, and naturally occurring substances showed the highest levels. Diethyl phthalate, currently listed for endocrine disruptor assessment, was the most abundant and widespread compound. Regarding aquatic environmental risk, only 15 of the identified compounds showed some occasional (usually in only 1 or 2 samples) high risk (RQ > 1).

Disclaimer/Disclosure:

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3.03.T-03 Advancing GC-APCI-TIMS-HRMS for Dioxins and POPs: A Comparative Evaluation of timsTOF Pro 2 and timsMetabo as Alternatives to Magnetic-Sector Instruments

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Magnetic-sector GC-HRMS remains the regulatory reference for dioxin quantification due to its high selectivity and sensitivity. However, its high cost and limited accessibility restrict global monitoring capacity. Recent advances in high-resolution GC-APCI-TIMS-HRMS provide a promising alternative. In previous work, we demonstrated that GC-APCI coupled to the timsTOF Pro 2 platform enables sensitive and confident determination of dioxins, and PCBs within a single analytical run. Building upon these validated results, the present study extends and enhances this framework by incorporating the next-generation timsMetabo platform, a small-molecule-optimized TIMS-QTOF system offering improved ion transmission and expanded mass/mobility range coverage.

A standardized GC-APCI workflow (35 min, 60 m × 0.25 µm column) was applied to both instruments to evaluate quantitation and identification performance for the 29 toxic dioxin, and PCB congeners, alongside additional POP classes. served as the reference platform, reflecting previously validated capabilities aligned with regulatory expectations for HRMS mass accuracy, retention-time stability, isotope-ratio agreement, MS/MS qualifier ion confirmation, CCS-based filtering, and Kendrick mass defect analysis. For the timsMetabo, data acquisition using the Mobility Range Enhancement (MoRE) mode, provided improved sensitivity and broader ion coverage. These enhancements particularly benefited congeners at the extremes of the mass range and species typically affected by transmission limitations, while also offering refined mobility separation and increased CCS precision.

Across both platforms, the workflow enabled robust quantitation of POPs in accordance with EPA 1613B and EN 1948 requirements. Detection limits for PCBs remained in the 10–20 ppt range, while timsMetabo allowed further improvements for several dioxin congeners. Real-sample applications (food and environmental matrices) confirmed the analytical reliability and showed the additional value of timsMetabo for challenging low-abundance analytes.

Overall, this evaluation demonstrates that GC-APCI-TIMS-HRMS is a viable and flexible alternative to magnetic-sector MS, with timsTOF Pro 2 providing a validated baseline and timsMetabo offering improved sensitivity and extended mass coverage for comprehensive multi-class POP analysis.

3.03.T-04 LC-IM-HRMS and Computational Methods for Characterization of Metal-Phytosiderophore Complexes in the Gas Phase

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Phytosiderophores (PS) released by graminaceous plants are root exudates that play a crucial role in the acquisition of micronutrients, particularly iron. Upon release into the rhizosphere, PS can also complex other metals present in the soil environment, potentially influencing their mobility and uptake, which may also lead to implications regarding safety of food and plant derived food additives.

Characterizing intact metal-PS complexes remains analytically challenging and so far, only a limited number of selected PS complexes have been investigated, primarily through targeted spectroscopy, mass spectrometry or electrochemical approaches. We will present a workflow using a mixed-mode stationary phase to separate 2'-deoxymugineic acid (DMA), mugineic acid (MA) and 3''-epi-hydroxymugineic acid (epi-HMA) and their complexes with Fe(III), Cu(II) and Ni(II). DTCCSN2 values were determined on two drift tube ion mobility systems in different labs using LC separation or direct infusion.

The DTCCSN2 values of the complexes were determined with excellent reproducibility (RSD $\leq$ 0.15%) across laboratories. These values were in linear relationship with the m/z of the investigated ligands and increased with the number of additional hydroxyl groups. Moreover, an inverse correlation of the ionic radii of the central atoms with the DTCCSN2 of the complexes was observed.

For better understanding correlations of metal-PS complex DTCCSN2 with other physical-chemical properties, such as the ionic radii of the central atom or the chemical structure of the organic ligands, DFT calculations were performed yielding optimized ion geometries and DTCCSN2 values predicted for all metal-PS compounds with excellent agreement to experimental values.

Disclaimer/Disclosure:

Eva Oburger (BOKU University, Institute of Soil Research) is acknowledged for providing phytosiderophore compounds.

3.03.P Advancing Environmental Monitoring through Ion Mobility Spectrometry coupled to HRMS: Innovative Strategies and Applications

3.03.P-Th036 Optimisation of a Liquid Chromatography-Trapped Ion Mobility-Time of Flight Mass Spectrometry Method for Suspect and Non-Targeted Analysis of Per- and Polyfluoroalkyl Substances in Fish

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Per- and polyfluoroalkyl substances (PFAS) are a large and chemically diverse group of persistent contaminants that accumulate in biota and remain analytically challenging, particularly when linear and branched isomers co-elute with other halogenated species in complex matrices such as fish. This study aimed to develop and optimise a liquid chromatography-trapped ion mobility-time of flight mass spectrometry method using parallel accumulation serial fragmentation (LC-TIMS-QTOF PASEF) in negative electrospray mode for PFAS suspect screening and collision cross section (CCS) determination, and to assess the transferability of CCS values across different ion mobility platforms. From an initial panel of 105 PFAS standards, 36 compounds spanning a wide range of polarity, mass, CCS and functional classes were selected as an optimisation set. Key TIMS parameters, including accumulation time, mobility ramp, ion transfer settings and number of PASEF scans per cycle, were systematically adjusted to balance sensitivity, ion mobility resolving power and CCS precision. Application of the final method to the full PFAS panel provided reproducible CCS values and enabled partial separation of branched and linear isomers in the mobility dimension. Two complementary travelling-wave ion mobility spectrometry (TWIMS) methods were then implemented on a Synapt G2-Si to cover the full structural diversity of the PFAS panel, enabling subsequent assessment of CCS transferability across platforms. CCS values obtained by TIMS and TWIMS agreed within approximately 2%, consistent with well-calibrated ion mobility platforms. The resulting PFAS CCS database, combined with curated suspect lists, a fully vendor independent data processing workflow and an ion-mobility-based confidence framework, was applied to support non-targeted PFAS annotation in fish extracts. In this context, CCS values and mobility trends helped refine candidate lists and uncover PFAS beyond routine monitoring panels, including emerging and under-characterised compounds relevant for exposure assessment and future environmental biomonitoring.

Disclaimer/Disclosure:

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nor the granting authority can be held responsible for them. The authors thank Wellington Laboratories, IFREMER and BRGM for providing PFAS standards.

3.03.P-Th037 Closing Sensitivity Gaps Across the Mass Range: Evaluation of a new Trapped-Ion-Mobility- QTOF MS for Emerging Contaminants Screening

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The accurate characterization of emerging contaminants (ECs) in complex environmental and biological matrices is often limited by their vast number, trace concentrations and severe matrix interferences. Highly sensitive, multidimensional LC-HRMS workflows are required to reduce false negatives and enable comprehensive identification, particularly for analytes in the low (<200 Da) and high (>750 Da) m/z ranges, where conventional LC-HRMS platforms often suffer from reduced ion transmission and coverage. In this study, we evaluate a new small-molecule-oriented LC-TIMS-QTOF platform (timsMetabo) for targeted and untargeted screening of ECs and PFAS in wastewater, surface water and biota. A complex standard mixture containing >400 contaminants and a set of real samples were analyzed on both a timsTOF Pro 2 and timsMetabo. We specifically investigated the impact of key hardware and acquisition innovations of timsMetabo, like the Mobility Range Enhancement (MoRE) mode, an improved high-precision quadrupole and the Athena Ion Processor (AIP) system, on ion sensitivity and mass/mobility range coverage. MoRE exploits the full length of the dual TIMS tunnel by performing ion accumulation and mobility separation in successive stages, extending the effective mobility range per scan and increasing ion throughput, particularly in the low-m/z region. The AIP, an additional RF/DC ion-optical stage downstream of the collision cell, dynamically optimizes ion transfer to the TOF analyzer across the full mass range, while the redesigned TIMS funnel increases ion capacity and transmission. For 315 ECs monitored across both platforms, timsMetabo delivered systematically higher signal intensities, with gains up to eightfold below m/z 300, an m/z region containing 60% of the investigated analytes. In complex wastewater and biota extracts, these improvements translated into higher feature counts, improved signal-to-noise ratios and richer MS/MS spectra, resulting in an increased number of confidently identified compounds and a reduced likelihood of false negatives across the mass range. Overall, timsMetabo operated with MoRE provides improved sensitivity and extended low-mass coverage compared to the previous generation platform. The resulting enhancement in data quality and depth strengthens LC-TIMS-HRMS workflows for environmental monitoring, supporting more comprehensive and reliable identification and prioritization of contaminants of environmental and toxicological concern.

3.03.P-Th038 Spatial mapping of PFOS and PFOA reveals differential bioaccumulation patterns in zebrafish via DESI-MSI with single- and multi-pass ion mobility separation

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Introduction:

A toxicological concern of PFAS chemicals is their ability to bioaccumulate, leading to chronic exposure even at low environmental concentrations. Mass spectrometry imaging (MSI) enables the mapping of molecules directly in tissues. Here we use desorption electrospray ionization (DESI) combined with a Q-ToF mass spectrometer combined with ion mobility to evaluate the distribution of PFOS and PFOA in zebrafish exposed in PFAS-spiked water.

Methods:

Zebrafish were divided into 3 groups, 1 male and 1 female per group. They were placed in 2L plastic tanks filled with 1L of water. The groups were: 1) Control, 0.1% DMSO; 2) PFOA 10 µM; 3) PFOS 10 µM. Water was changed daily for seven days. Fish were sacrificed by thermal shock and embedded in 3% CMC for spatial analysis.

Results and Discussion:

Zebrafish were cryo-sectioned onto standard glass slides at a thickness of 18 µm and stored at -80°C until analysis using a DESI XS source mounted on a SELECT SERIES Cyclic IMS mass spectrometer. The DESI MSI analyses were performed in single pass IMS untargeted full scan MS mode to enable detection of PFAS. In negative mode, the ion mobility of PFAS species can be easily distinguished from singly charged tissue endogenous molecules, which have a longer drift time compared to PFAS ions that increase the peak capacity

for PFAS detection. PFOS was detected as its intact [M-H]⁻ ion, whereas PFOA fragmented with the loss of CO₂, giving [PFOA-CO₂-H]⁻ as the most abundant peak. PFOS was distributed throughout the zebrafish, but it was primarily accumulated in the gills, digestive system and surrounding the eye, whereas PFOA was mainly localised in the gills and less into the digest organs. Using a TWIMS cyclic device, following several passes of ion mobility separation, it was possible to optimise the number of passes to differentiate the linear and branched PFOS chemical standard that was used in the water spike. The IMS sequence was then used for the DESI MSI on the zebrafish enabling visualisation of the different isomers.

Conclusion

Using the IMS single pass, untargeted MSI experiments were performed to be able to localize PFOS and PFOA-CO₂ in zebrafish and evaluate their bioaccumulation. It was possible to visualize the distribution of metabolites and lipids within the same experiment. Furthermore, using the IMS multi-passes functionality within the cyclic IMS, linear and branched PFOS isomers were separated and localized in the contaminated zebrafish.

3.03.P-Th039 Illustrating the Use of Cyclic Ion Mobility to Enhance Specificity for branched-PFAS

Isomer Analysis

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Per- and polyfluoroalkyl substances (PFAS) are widely used chemicals with significant environmental and health concerns, particularly due to the complexity introduced by branched isomers such as those of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). This study demonstrates the application of LC-cIM-MS using the SELECT SERIES™ Cyclic™ Ion Mobility Mass Spectrometer to resolve and characterize PFAS isomers with enhanced specificity.

Travelling wave ion mobility (TWIM) collision cross section (CCS) values were measured for PFAS standards and showed excellent agreement (Δ CCS <0.7%) with drift tube and trapped ion measurements, confirming CCS reliability for transferable PFAS analysis. Chromatographically coeluting branched isomers were successfully separated using ion mobility-enhanced peak capacity, and multipass cyclic IMS improved resolution from R~65 to R~145, delivering an eight-fold increase in peak-to-peak resolution (RsP-P). Single-component fragmentation spectra enabled structural elucidation of branched PFOS byproducts, while PFOA isomer dimers provided additional specificity.

These findings highlight LC-cIM-MS as a robust, non-targeted screening approach for confidently identifying known and unknown PFAS isomers, supporting environmental monitoring and exposomics studies where differentiation of isobaric and isomeric species is critical.

3.03.P-Th040 Conformational Effects of Monovalent Cations on PFCA Homodimers Investigated by IMS-MS/MS and Theoretical Calculations

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Per- and polyfluoroalkyl substances (PFAS) are contaminants of emerging concern requiring non-targeted screening to identify novel species. When analyzed by liquid chromatography coupled to ion mobility-mass spectrometry (LC-IMS-MS), some PFAS, such as perfluorocarboxylic acids (PFCAs), form noncovalent proton-bound homodimers, raising questions about the conditions under which such aggregates form, their structures, and how cation binding influences their conformation. While proton-bound dimers have been previously characterized, the structural implications of replacing the proton with larger monovalent cations remain unclear. To address this, PFCA homodimers bound to Li⁺, Na⁺, K⁺, and Cs⁺ were analyzed using three IMS platforms: drift-tube IMS, trapped IMS, and traveling wave IMS (DTIMS, TIMS, TWIMS). Experimental collision cross-section (CCS) values were compared across instruments, and relative binding strengths were assessed through energy-resolved collision-induced dissociation (CID, also known as breakdown curve experiments). Three-dimensional structures were generated and evaluated by predicting CCS values via the trajectory method in nitrogen. Fragmentation pathways were examined to further support structural hypotheses.

CCS measurements for all cation-bound dimers were consistent across instruments (within 2% deviation) and increased with cation size, though the magnitude of this increase depended on chain length. This behavior suggests that CCS differences cannot be explained solely by mass increase and instead suggests structural rearrangements of the monomeric chain after cation binding. Quantum-chemistry results indicate that

fluorinated chains tend to fold toward the cation coordination site, similar to proton-bound dimers, at least in the case of sodium (the structures with the other cations still need to be computed). Breakdown curves and identified fragmentation channel support the proximity of a fluorine atom in agreement with theoretical modeling.

Overall, these results suggest that PFCA chains remain in close contact upon cation binding and that ion identity influences aggregate structure and stability. Final V_{50} values (acceleration voltage used during CID, which induces 50% fragmentation of the initial intensity of the precursor ion) and additional theoretical structural determination will further clarify the relationship between cation size, binding energetics, and dimer conformation.

Disclaimer/Disclosure:

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3.03.P-Th041 Atmospheric PFAS Partitioning and Source Attribution Using a Trajectory-Informed Non-Targeted Approach: Insights from Seoul

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Poly- and perfluoroalkyl substances (PFASs) are synthetic chemicals widely used in industrial and consumer products. Their persistence, resistance to degradation, and potential toxicity raise concerns regarding their environmental fate and transport. This study investigates atmospheric PFAS concentrations in Seoul, Korea, focusing on their gas/particle partitioning behavior. A total of 21 atmospheric samples were collected from February 10 to June 20, 2024, using a High-Volume Air Sampler. Particulate matter was captured on Quartz Fiber Filters (QFF), while gas-phase compounds were collected using polyurethane foam (PUF)/activated carbon felt (ACF)/PUF blocks. The samples were analyzed by HPLC-MS/MS. Total PFAS concentrations ranged from 8.891 to 168.0 pg/m³ (mean: 51.68 pg/m³, median: 30.13 pg/m³). Overall, short-chain PFASs (C₇) exhibited higher concentrations than long-chain PFASs (C₂₈). For example, the concentrations of PFPeA (0.9160 pg/m³) and PFHxA (0.3095 pg/m³) were higher compared to PFDA (0.0767 pg/m³) and PFUnDA (0.0650 pg/m³). Phase-distribution analysis showed that PFBA (74%) and PFBS (99%) were predominantly in the gas phase, whereas PFOA (53%) and PFOS (80%) were more commonly found in the particle phase. The gas/particle partitioning indicated that short-chain PFASs are more volatile and likely to undergo long-range transport, whereas long-chain PFASs exhibit a stronger tendency to adhere to particulate matter and deposit locally.

In addition to target analysis, non-target screening (NTS) was conducted using high-resolution mass spectrometry to explore unknown PFASs in the atmosphere. Orbitrap-MS data were processed through the FluoroMatch Modular workflow, and 43 features with confidence level C or higher were identified. These features include many compounds that are not reported in existing databases, suggesting the presence of novel or transformation-derived PFASs. Multivariate analysis using Partial Least Squares Discriminant Analysis (PLS-DA) enabled differentiation of chemical profiles by air mass origin and revealed source-specific PFAS candidates. This integrated approach combining targeted quantification and NTS-based discovery highlights the complexity of airborne PFAS mixtures and underscores the necessity for comprehensive monitoring strategies.

3.03.P-Th042 Enabling In Vitro Studies of Difficult to Test Petroleum-Derived Unknown or Variable Composition, Complex Reaction Products, or Biological Materials with Nanoemulsion-Based Delivery

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Refined petroleum products (RPPs) are classified as UVCBs (Unknown or Variable composition, Complex reaction products, or Biological materials) and contain diverse polycyclic aromatic compounds with varying hazardous properties. Although these UVCBs are regulated under EU REACH, most have limited compositional and toxicological data for registration. Efforts to use read-across to meet data requirements have been hampered by challenges in establishing chemical similarity and characterizing their compositional variability. To address these gaps, we developed a novel nanoemulsion-based delivery system, adapted from pharmaceutical formulations, to enable cell-based testing of hydrophobic petroleum substances without solvent extraction. Our objective was to compare toxicological responses of light versus heavy petroleum products and to evaluate

delivery performance in vitro. Stable nanoemulsions of light and heavy RPPs, crude oil, and vegetable oil were prepared using 2% Pluronic F-127 as surfactant under controlled shear conditions. EA.hy926 human endothelial-like cells were exposed to 1-50% (v/v) nanoemulsions for 120 hours, and cytotoxicity and metabolic activity were quantified via luminescent viability assays. Post-exposure media and lysates were analyzed by ion mobility spectrometry–mass spectrometry (IMS–MS) to characterize hydrocarbon profiles and cellular uptake. The Pluronic-stabilized nanoemulsions remained stable and reproducibly delivered both light and heavy RPPs to cells. Light aromatic RPPs (C9-C16 hydrocarbons) caused concentration-dependent decreases in viability, indicating higher cytotoxic potential. In contrast, heavy hydrogen-treated RPPs (C20-C50 saturated hydrocarbons) elicited no cytotoxicity across comparable exposures. These divergent cellular responses align with expected in vivo hazard profiles. IMS–MS analysis confirmed differential composition and bioavailability between products. This work establishes a feasible and reproducible nanoemulsion-based system for in vitro assessment of complex petroleum UVCBs, overcoming solubility and delivery barriers typical of hydrophobic substances. The approach enables mechanistic evaluation of toxicity, supports read-across justification, and advances hazard characterization of petroleum-derived mixtures under REACH and related regulatory frameworks.

3.03.P-Th043 Nontarget Identification of Contaminants of Emerging Concern in Campus Indoor Dust Using Ion Mobility High-Resolution Mass Spectrometry

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Contaminants of emerging concern (CEC) are increasingly reported in indoor environment, where people spend most of their time, serves as a major reservoir for contaminants through the accumulation of indoor dust. Industrial additives such as organophosphate flame retardants, alternative plasticizers, and fluorescent brighteners are of particular interest due to their potential human toxicity and structural diversity. However, their identification in complex matrices is hindered by limited availability of reference standards. Ion mobility high-resolution mass spectrometry provides an additional dimension of confidence by incorporating collision cross section (CCS) values.

In this study, indoor dust was collected from multiple functional zones across a university campus in North China. A hybrid screening workflow combining diagnostic fragment filtering with suspect and nontarget screening was implemented to maximize analytical coverage. Automated processing integrated peak detection and alignment, reference databases matching, and in silico fragmentation matching, while ion mobility data enabled calculation of experimental CCS values.

A total of 81 CECs were identified, including 41 confirmed with reference standards and semi-quantified. An additional 40 CECs were identified and assigned at Level 3 or above with supporting evidence. These CECs showed high detection frequencies across all samples, with potential co-occurrence patterns among chemicals sharing similar industrial applications such as antioxidants, food-packaging additives, and polyvinyl-chloride-related additives. Correlations between parent compounds and their corresponding transformation products possibly suggesting common emission sources or in situ degradation. CCS versus m/z trendlines were established for all chemical classes (187 ions across adducts). Distinct class-dependent behaviors were observed, including a pronounced power regression trend for plasticizers and more dispersed distributions for bulkier fluorescent brighteners, reflecting differences in gas-phase structure. Overall, integrating collision cross section trendlines enhanced identification confidence and provided an effective filter for excluding false positives, supporting rapid discovery of unknown indoor dust contaminants.

3.03.P-Th044 Uncovering Chemical Signatures in PM10 Aerosols: Insights from Six Northern Italian Cities Across Two Seasons

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Atmospheric aerosols, especially the PM10 fraction, are complex matrices composed of inorganic particles, biological material, and thousands of organic compounds originating from both natural and anthropogenic sources. In recent years, mass-spectrometry-based approaches have revealed the presence of numerous emerging contaminants within airborne particulate matter, including transformation products, industrial chemicals, and previously unreported molecular features. Understanding how these chemical signatures vary across locations and seasons is essential for characterizing air quality and identifying potential exposure-relevant compounds. In this study, we investigated whether reproducible chemical patterns can be observed in PM10 aerosols

collected in six cities in Northern Italy, representing three types of environments: two alpine, two urban, and two port areas. For each city, winter (December) and summer (July) samples were collected to assess both spatial and seasonal variability.

After extraction, samples were analyzed using a HPLC-TIMS-TOF system (timsTOF Pro 2, Bruker), leveraging trapped ion mobility separation to improve resolution of isomeric species. Data alignment and feature annotation were performed in MetaboScape (Bruker) using MassBank and NIST databases, followed by blank removal, normalization, and additional processing in R.

Multivariate and unsupervised analyses (including UMAP, hierarchical clustering, and heatmaps) revealed clear grouping patterns. Samples clustered strongly by city and by season, indicating consistent local chemical signatures and pronounced intra-city seasonal shifts. UMAP projections highlighted well-defined separations among the cities as well as between winter and summer aerosols. Ongoing work focuses on identifying the molecular features that drive these differences, with the aim of pinpointing compounds that are characteristic, shared, or unique across environments.

Overall, this study provides an initial framework for characterizing spatial and seasonal variability in PM10 composition and supports the use of ion-mobility high-resolution mass spectrometry to explore emerging contaminants in atmospheric aerosols.

3.03.P-Th045 Assessing & Predicting the Fate of Veterinary Pharmaceuticals & their Transformation Products in Soils and Towards the Aquatic Systems.

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Pharmaceutical pollution in soil and aquatic ecosystems poses an increasing threat to environmental integrity, microbial diversity, and ultimately to human and animal health. Veterinary pharmaceuticals and their transformation products are of particular concern, as they enter soils through manure application and agricultural practices, subsequently migrating toward water bodies where they exert toxicity and selection pressure on microbial communities. Despite their importance, the environmental fate of many transformation products (TPs) remains poorly understood, limiting effective risk assessment and mitigation strategies.

This PhD project investigates the migration of Toltrazuril, a veterinary antiparasitic pharmaceutical, by assessing its fate across different environmental matrices (manure, soil, plants, and leachate). Additionally, it explores the plant metabolic response to Toltrazuril exposure. The project also integrates the use of TyPol, a compound classification tool, to enable a priori prediction of transformation products, their behaviour in soil, and their potential transfer to aquatic environments.

These objectives are addressed by (i) combining controlled semi-field degradation and plant uptake experiments, and (ii) conducting mesocosm dissipation studies. The samples are analysed using advanced LC-HRMS targeted methods alongside untargeted metabolomics.

Ultimately, this project aims to strengthen environmental risk assessment frameworks. While abundant information exists on pesticides in soil contamination, the data generated here will help fill the current knowledge gap regarding pharmaceuticals, thereby supporting both regulatory processes and agricultural decision-making.

3.04.A Advancing Understanding of Environmental Persistence of Chemicals

3.04.A.T-01 Biodegradation Testing Beyond Standardized OECD 301 Screening Methods

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Today persistence assessment relies on OECD testing guidelines that can be subdivided into two categories: stringent, but easy and rapid to perform (ready or inherent) screening biodegradability testing or complex and challenging higher tier simulation tests. In 2025 ECHA published the “Key areas of regulatory challenge” highlighting the existence of a methodological gap between biodegradation screening tests and higher-tier simulation tests.

This work introduces methodological advancements aimed at enhancing the reproducibility and understanding of screening biodegradation tests and addressing gaps between screening and simulation studies. Experimental efforts focused on three different aspects: firstly, optimization of the inoculum treatment to achieve more

reproducible results in OECD 301F or B methods. Secondly, the exploration of different inoculum to test ratios utilizing existing commercially available respirometric test set-ups. The conditions explored span from OECD 301 F/B conditions beyond those utilized for OECD 302 B protocol. Lastly, we investigated the use of microbial adaptation and enrichment experiments in combination with DNA sequencing to understand the role of microbial population in biodegradation tests. The experiments presented have been focusing mostly on water-soluble polymers, to assess the applicability of existing and optimized methodologies to this class of materials. For all tests protocols, testing times have been extended to reach biodegradation plateau.

Our findings highlight strengths and limitations of existing OECD 301 test methods and provide experimental insights on how to further develop biodegradation testing using respirometric methods and microbial cultivation to achieve affordable and reliable biodegradation results for chemicals and water-soluble polymers.

3.04.A.T-02 Regulatory degradation testing of difficult-to-test substances

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Persistence assessment has a vital role in evaluating long-term risks of chemicals in the environment and human health. It is acknowledged that the testing and the identification of persistent chemical substances, is often challenging and time consuming which hampers efficient identification and management of hazardous substances. These challenges may result from specific physico-chemical properties of the substance leading to difficulties in conducting standard, screening and/or simulation tests relevant for the regulatory assessment or from the complexity of the substance composition causing specific challenges in analytics and interpretation of the results. Biodegradation testing of such substances may require specific precautions or even modifications or adaptation of the standard test guideline methodologies to obtain data adequate for regulatory purposes.

Available guidance to support regulatory degradation testing and persistence assessment of chemicals with difficult-to-test properties and/or of complex composition needs updating. In this presentation, the outcome of the literature review for available modifications in the standardised biodegradation test methods identified for potential regulatory use will be presented.

Four benchmark publications and reports were reviewed and used to list the properties of difficult-to-test substances discussed in each of these publications. The challenges associated with testing, sample processing, chemical analysis, and data treatment in standard biodegradation studies with difficult-to-test substances were reviewed and extracted from the literature. Then detailed targeted literature search was conducted in three databases for scientific literature to identify, as much as possible, all the available relevant publications which propose adapted methodologies or recommendations (e.g. for test procedures or data treatment) for testing the biodegradability of difficult-to-test substances.

The methodological modifications proposed in those publications, together with the challenges they are meant to address are being compiled. The regulatory relevance of those modifications as well as the practical applicability of proposed changes in routine laboratory work will then be assessed. The results of this literature search including modifications identified for potential regulatory use will be collected and presented at SETAC.

3.04.A.T-03 High-Throughput Miniaturized Biotransformation Screening: Method Validation and Robustness 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 commonly evaluated using outdated, time-intensive, and resource-heavy methods. The European Chemicals Agency recently addressed this issue by describing a gap between generic screening methods and complex higher-tier simulation tests, and advocates for high-throughput methods for the assessment of degradation potential of substances under stringent and/or relevant conditions.

To address this, we developed a miniaturized 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 primary biotransformation, while avoiding the “microbial lottery”. 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.

Previous experiments validated biotransformation behaviour between the 24 well-plate format and 50mL glass control vessels by applying a library of 33 pesticides and pharmaceuticals. We aimed to expand this validation by analysing a mix that covered a larger chemical space and containing 189 chemicals, consisting of pharmaceuticals, household and personal care products, and pesticides. Good correlation between the scales confirmed the similarity in biotransformation behaviour.

To assess the influence of different activated sludges sources on biotransformation rates, we conducted experiments using sludges from multiple wastewater treatment plants. Approaches to minimize variance or increase robustness included normalization by total suspended solids, universal benchmarking, and categorization using benchmark compounds. On average, normalization using total suspended solids reduced multiplicative variability between sludges.

These results validate the use of the method for a larger chemical space than previously tested. In addition, method robustness was increased by reducing the variance between biotransformation rates. Using the method, a high-throughput assessment of degradation potential of substances in activated sludge can be obtained.

3.04.A.T-04 Linking Assimilable DOC Levels to Microbial Metabolic Strategies and OMP Persistence in Groundwater

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Groundwater microbial communities rely on dissolved organic carbon (DOC) as their primary energy source, yet the role of assimilable DOC (aDOC) in shaping biodegradation processes remains unclear. In oligotrophic conditions, where aDOC typically ranges between 0.01 to 0.1 mg/L, carbon scarcity may constrain metabolic activity and thereby limit the biotransformation of organic micropollutants (OMPs). Previous DOC-amendment studies have reported conflicting outcomes, highlighting the need to clarify how aDOC levels influence microbial metabolism and OMP biotransformation capacity in oligotrophic conditions. This research provides a mechanistic assessment of how the amount of aDOC affects microbial carbon utilisation strategies and OMP biodegradation kinetics in groundwater.

To investigate this, aerobic groundwater microcosms were amended with different aDOC concentrations spanning four orders of magnitude (0.001-10 mg/L). Biotic and abiotic microcosms were spiked with a 22-compound OMP mixture (~800 ng/L per compound). Microbial respiration, biomass production, and OMP concentrations were monitored over 100 days, and first-order biodegradation kinetics were derived after correcting for abiotic losses.

Results show that carbon availability strongly influences microbial metabolic strategies: low aDOC concentrations enhanced bacterial growth efficiency, while high concentrations promoted respiration at the expense of biomass synthesis. OMP biodegradation peaked at moderate aDOC levels (0.01–0.1 mg/L), with the greatest number of compounds exhibiting significant biotransformation. In contrast, both carbon scarcity and carbon saturation suppressed biotransformation—either by limiting microbial energy or by inducing substrate competition and potential functional shifts.

These results demonstrate that aDOC levels influence microbial metabolic strategies and, consequently, OMP biotransformation kinetics in groundwater. This mechanistic link between substrate availability, microbial metabolism, and biotransformation rates helps explain variability in groundwater persistence outcomes and provides insights relevant for refining biodegradation test conditions in groundwater-relevant systems.

3.04.B Advancing Understanding of Environmental Persistence of Chemicals

3.04.B.T-01 Effect of Inoculum Location on Marine Biodegradation Kinetics

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Marine pollution is of growing concern and produced water from offshore oil platforms is an example of a high-volume complex mixture being discharged to the sea. Biodegradation testing of chemicals for persistence

assessment is a crucial part of chemical regulation, however studies on differences and variation in biodegradation kinetics in marine environments are lacking. This study aims to 1) quantify the difference in marine biodegradation kinetics between harbors and open water (expected high preexposure compared to low preexposure), 2) quantify the variation in marine biodegradation kinetics between different open water sampling locations at expected low pre-exposure and across a salinity gradient) and 3) investigate if the microbial composition of the inoculum samples and composition during incubation can explain differences or similarities in biodegradation kinetics for different sampling locations. Seawater samples were collected in harbors and open water (6-8 km from shore) at three sites across Denmark. Test systems contained produced water diluted 1:200 in seawater (biotic test systems) and were incubated 1-60 days at 10°C. Solid Phase Microextraction coupled to GC-MS was used to determine primary biodegradation kinetics of 27 petrochemicals in the produced water based on the chromatographic peak area ratios (biotic test systems/abiotic controls). The biodegradation half-times of the 27 petrochemicals were on average 2.9-3.6 times shorter in harbor samples compared to open water samples, in agreement with higher initial microbial abundance in the harbor samples and higher expected pre-exposure. The variation in half-times of 27 petrochemicals between the three open water samples was on average a factor 1.2-1.9. Similarity in inoculum sample location and salinity were reflected in microbial composition for two sites, however, biodegradation kinetics correlated better with expected pre-exposure than similarity in geographic sampling location and salinity. Alignment of microbial composition development during incubation with biodegradation kinetics will be presented at the conference. In conclusion, biodegradation half-times were markedly shorter when using inoculum water from harbors compared to open sea. For conservative persistence assessment, these results support that inoculum generally should be sampled from less exposed locations. However, the optimal sample location ultimately depends on the specific purpose of the biodegradation test.

Disclaimer/Disclosure:

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3.04.B.T-02 Higher Microbial Diversity Enhances the Extent and Rate of Organic Micropollutant Biodegradation Under Aerobic and Nitrate-reducing Conditions

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Organic micropollutants (OMPs) are constantly released in the environment, where their fate depends on both molecular properties and biogeochemical conditions. Depending on site-specific environmental and microbial conditions, OMPs might persist or transform into transformation products (TPs), with distinct properties and potential risks. In our study, we investigated how microbial diversity and redox conditions influence the biodegradation and transformation of 20 spiked OMPs. Microbial communities with three different levels of diversity were created using a solid-phase dilution-to-extinction approach, and after regrowth, were incubated under aerobic and nitrate-reducing conditions. During incubation, biodegradation of the spiked OMPs and TP formation were monitored through target and non-target analyses. Our results show that higher-diversity communities achieved greater biodegradation performance, with significant biodegradation of 15 OMPs under aerobic and 5 OMPs under nitrate-reducing conditions. Higher diversity communities also showed higher OMP degradation rates and greater numbers of total and later-generation TPs. Low-diversity communities showed limited biodegradation, revealing a threshold in microbial diversity below which the biodegradation potential is affected. Redox conditions strongly influenced the fate of OMPs. Aerobic conditions were superior, while several OMPs were persistent only under nitrate-reducing conditions. Redox-specific TPs and TPs with potential endocrine-disruptive effects were identified through in-silico methods. These results demonstrate that both microbial diversity and redox conditions significantly influence environmental persistence and transformation of OMPs. However, standardized tests for persistence assessment, like OECD 308 and 309, overlook these factors, potentially leading to erroneous conclusions. Incorporating microbial diversity and redox conditions into regulatory biodegradation testing could improve the reliability and accuracy of persistence assessments and chemical risk evaluations.

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3.04.B.T-03 Explaining the Variability in Micropollutant Biodegradation Across Aquatic Ecosystems Through Microbial Respiration

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The significant variability observed in biodegradation rates across aquatic environments is a major source of uncertainty in assessing the persistence of micropollutants. While some parameters such as total organic carbon have been identified as influences on biodegradation rates, most of its variability remains unexplained. In a previous study, we observed significant positive relationships between biodegradation rates and water-sediment BOD for 23 of 34 tested compounds across 10 sites in a river system. This relationship can be mechanistically explained by higher BOD indicating increased microbial respiration and metabolic activity, which provides more opportunities for co-metabolic degradation. Here, we assess this potential relationship between biodegradation and respiration rates across 7 rivers using a modified OECD 309 incubation test alongside respiration tests on the same samples. Of the 50 assessed micropollutants, 24 showed a significant ($p < 0.05$) positive relationship, 2 showed a significant negative relationship, and 24 showed no significant relationship between biodegradation and respiration rates. Normalizing the biodegradation rate constants, k , by the highest k value for each compound to produce values between 1 and 0 enabled comparisons of overall biodegradation rates across sites. Among the 7 sites, three exhibited substantially higher respiration rates, and all three had generally higher normalized k than the four sites with lower respiration rates. This difference was significant for all pairs except one. After normalizing respiration rates in the same way as k , correlation analysis of normalized k against normalized respiration rates across the entire dataset showed a highly significant positive relationship ($p = 2.2 \times 10^{-16}$). The ρ value was 0.48, indicating that microbial respiration explains a modest but meaningful portion of biodegradation variability among rivers. Additionally, biphasic kinetics with rapid initial degradation that slowed considerably after approximately 50 hours were observed for certain compounds at low respiration sites, possibly indicating that biodegradation rates depend on compound or natural substrate concentrations. Overall, these findings demonstrate a significant positive relationship between microbial biodegradation and respiration rates for about half of the tested compounds and explain a considerable portion of the variability in biodegradation across the studied sites environments.

3.04.B.T-04 Tracing Organotin Degradation Using Multi-Isotope Labelling in Marine Sediments: The Role of Microbial Communities and Sediment Properties

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Tributyltin (TBT), formerly one of the most widely used biocides in antifouling paints, has been globally banned for almost two decades due to its extreme toxicity to non-target organisms. Despite the ban, TBT is still detected in many coastal environments, raising concerns about whether recent contamination originates from the illegal use of antifouling coatings or from the remobilisation of “legacy” TBT stored in historically polluted sediments. Understanding the processes that control the fate, persistence and transformation of TBT in sediments is therefore essential. Although degradation rates clearly differ among sediments, the reasons for this variability remain poorly understood. In particular, the relative importance of abiotic processes such as sorption or mineral-mediated reactions versus biotic degradation driven by benthic microbial communities is still insufficiently resolved.

In this study, we investigated the mechanisms and kinetics of TBT degradation and its stepwise transformation through DBT and MBT to inorganic Sn in a range of marine sediments subjected to oxic and anoxic conditions. Sediments were spiked with isotopically labelled organotin species (^{119}Sn -TBT, ^{119}Sn -DBT and ^{119}Sn -MBT) to enable precise tracking of each individual degradation step over a 180-day incubation. Concentration changes were monitored to determine degradation pathways. The structure of benthic microbial communities was characterised by 16S rRNA amplicon sequencing at multiple taxonomic levels (phylum to family), performed on oxic and anoxic sediment samples at the start and after 50 days of incubation to assess community shifts. Functional genes associated with TBT degradation were quantified by qPCR, while sediment properties, including particle size distribution, mineralogical composition, carbonate content, multielement composition and total organic carbon, were measured to evaluate their role in transformation processes. Based on the obtained

results, kinetic rate constants were calculated for each degradation step and the overall mechanism of TBT transformation was established. Statistical analyses revealed how the composition of the benthic microbial community and key sediment characteristics influence degradation kinetics, thereby determining the environmental persistence of TBT in contaminated marine sediments.

Disclaimer/Disclosure:

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3.04.P Advancing Understanding of Environmental Persistence of Chemicals

3.04.P-Tu158 Persistence Directed Testing of Chemicals in Mixtures

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Test data for persistence assessments are lacking for many of the chemicals that are produced, imported, or used in the European Union. Typically, standardized tests are conducted as single substance tests aimed at demonstrating biodegradability, which directs a disproportionate fraction of an extensive testing effort on a large number of degradable chemicals. It is much more crucial to identify persistent chemicals since they can cause severe and long-lasting pollution.

In this project we develop and apply a persistence directed testing strategy to identify persistent chemical structures in an efficient way rather than confirming the biodegradability of chemicals. The strategy consists of persistence testing of chemicals in mixtures in aqueous simulation type tests. The absence of primary biodegradation is used as evidence of persistence.

Three rounds of testing will be conducted in the project. The first round was conducted with two mixtures containing 15 and 46 chemicals at constituent concentration levels of 1 and 5 µg/L. The mixtures showed no inhibition in a biotox test up to constituent concentrations of 10 µg/L. The tests were conducted with surface water from a Swedish stream draining forest and marsh areas with a high organic and low nutrient content (TOC: 24 mg/L and NOx: 52 µg N/L). Triplicate biotic and abiotic test systems were prepared in 1 L bottles for each mixture and concentration level, incubated at 12°C, and sub-sampled on day 7, 14, 21, 28, 40, 50 and 60. Samples were analyzed with LC-MS/MS. This first test round provides primary biodegradation kinetics for some chemicals and a list of chemicals that were not degraded. The non-degraded chemicals will be included in the second and third test round to confirm their persistence.

3.04.P-Tu159 Assessing fungal derived enzymes for screening biodegradability of plant protection products

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Plant protection products are widely used to manage weeds, pests and diseases threatening stable food crops. To avoid the accumulation of plant protection products in the environment, the persistence of the respective active ingredients (AI) is evaluated under standardised OECD tests. The OECD301 is used to identify degradable AIs by microbial breakdown under aerobic conditions. This approach allows screening multiple AIs and ensures compliance with AI persistence requirements in the EU. The OECD 301 test is resource-intensive and requires high active ingredient concentrations. A simpler, faster test would be useful for earlier screening of pesticide degradability during AI development.

This study aims to develop a fungal derived crude enzyme extracts (CEEs) based degradability screening assay as a simple and faster complementation to OECD301. CEEs, rich in laccase and lignin peroxidase, catalyse the oxidative transformations of phenolic compounds and are known for their broad substrate specificity, stability and capacity for breaking down of active ingredients of plant protection products.

CEEs were produced through a 14-day submerged fermentation of the fungal strain *Phlebia radiata* in a kraft lignin-based medium. The efficacy of these CEEs in degrading pesticides was evaluated over 9 days at 20 °C

and 120 RPM. Samples were analyzed for pesticide concentration and specific enzyme activities of laccases and lignin peroxidase. The tested pesticides and transformation products are: 2,4-D, 2,6-dichlorobenzamide, aminomethylphosphonic acid, atrazine, bromoxynil, chloridazon, mecoprop, imidacloprid, metolachlor ESA, and metolachlor OA.

Laccase activity was stable, ranging from 1.4 to 2.0 U/mg protein, and lignin peroxidase activity varied between 1.7 to 2.5 U/mg protein. Notably, a reduction of over 30% in concentration was observed for AMPA, atrazine, bromoxynil, and imidacloprid, while other compounds exhibited decreases of approximately 15-20%. These findings demonstrate the potential of CEEs to facilitate the oxidative degradation of pesticides. Future validation with a wider range of compounds will enhance the robustness of this rapid fungal exoenzyme assay, positioning it as a valuable tool for preliminary screening of pesticide biodegradability and prioritization for further testing.

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3.04.P-Tu160 Exploring the Relationship between Microbial Biodiversity and Pollutant Biodegradation in Rivers

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Rivers are critical ecosystems that provide drinking water, support biodiversity, and regulate biogeochemical cycles, yet they are increasingly burdened by complex mixtures of micropollutants from urban, agricultural, and industrial sources. Microbial communities mediate the natural attenuation of these contaminants, but the extent to which biodiversity underpins biodegradation capacity across diverse environmental settings remains unresolved. We designed a comparative study of 18 river segments spanning five European countries to explore the relationship between microbial biodiversity and pollutant biodegradation. Biodegradation assays were conducted for 97 micropollutants, including pharmaceuticals, pesticides, and industrial chemicals, while microbial community profiles were generated using metagenomic sequencing. Central to our framework is the functional redundancy hypothesis, which posits that diverse microbial assemblages can sustain pollutant attenuation despite taxonomic turnover. By integrating taxonomic and functional diversity metrics with compound-specific biodegradation rates, this study aims to disentangle whether redundancy provides resilience across chemical classes or whether unique taxa and gene repertoires are required for specific degradation pathways. Situated within the context of escalating chemical pressures on European freshwater systems, anticipated outcomes will advance ecological theory on biodiversity–function relationships and inform strategies for incorporating microbial metrics into environmental risk assessment, river basin management, and water quality policy.

3.04.P-Tu161 What Drives Variability in OECD TG 309 Studies? A Data-Driven Evaluation of 176 Surface Water Simulation Tests

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The OECD Test Guideline (TG) 309 ‘Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test’ is a cornerstone method for assessing the degradation of chemicals under environmentally relevant conditions. Despite its critical role in regulatory aquatic hazard and risk assessments, including PBT assessment, TG 309 studies are complex, technically demanding, and can exhibit substantial variability in outcomes (e.g.

half-lives).

High variability in reported half-lives has been attributed to multiple factors in the scientific literature, including inoculum properties, surface water characteristics, and differences in experimental design (e.g., test concentration and system configuration). While individual sources of variability have been explored in isolation, their relative influence and combined impact remain poorly understood, particularly in studies conducted for regulatory purposes.

This work aimed to identify and rank the key factors - both substance-specific and system-related - that influence outcomes in OECD TG 309 studies. A comprehensive database of 176 independent TG 309 studies was compiled, including data sourced from the European Chemicals Agency (ECHA) dissemination dossiers and European Food Safety Authority (EFSA) Risk Assessment Reports. Statistical analyses have been performed to examine relationships between test system parameters, chemical properties, and observed degradation half-lives, with the goal of quantifying which factors contribute most to observed variability. Initial results were presented at the SETAC Europe 35th Annual Meeting, and this presentation will report the final outcomes and interpretations from the completed analysis.

The insights gained from this work are expected to clarify the primary drivers of variability in TG 309 outcomes. This improved understanding could support the optimisation of the TG 309 protocol, enhance its reliability and reproducibility, and ultimately strengthen its application in regulatory hazard and risk assessments.

3.04.P-Tu162 Round Robin Test to prepare for consolidation of tests for ready biodegradability according to OECD 301/310

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Persistence is a key component of the regulatory assessment, including the identification of substances of very high concern (SVHC) under European Chemicals Regulation (REACH; regulation (EC) Nr. 1907/2006). As a first step, Ready Biodegradability Tests (RBTs) are used to determine whether a substance can be exonerated from the concern of persistency, or if higher Tier simulation degradation tests are required for decision making on persistency. Although the RBTs are generally considered equivalent for verification purposes, they differ in some respects of the individual testing protocols.

The intention of this R&D project is therefore to prepare the first steps towards consolidation of the OECD guidelines for RBTs, i.e. to harmonise the test conditions as far as possible, taking into account the technical progress since the last major guideline revision in 1992.

In this context, a round robin test is being conducted with 12 GLP laboratories from across the OECD region. The test design involves performing a slightly modified OECD 301F test with additional DOC-measurement at the end and the beginning of the test (similar to OECD 301C). Three test substances, potassium hydrogenphthalate, diisobutyl hexahydrophthalate and acesulfame are tested, which cover the range of assessment criteria from “not readily biodegradable” to “readily biodegradable”. Aniline was chosen as a reference substance, with the aim of putting a very well-established reference substance to the test, for which historical data sometimes show differences.

The statistical analysis of the tests is to have a special significance. Therefore, two independent repetitions are conducted with different batches of inoculum. This enables to calculate intra- and interlaboratory variability according to ISO 5725-2. In combination with an extended inoculum analysis, which is intended to complement the determination of the CFU, this offers the possibility of illuminating the reproducibility of the results across geographical boundaries and different technical capabilities.

3.04.P-Tu163 Positive Controls for OECD Biodegradability Guidelines: Rethinking Robustness

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Biodegradability is crucial for environmental assessment. OECD guidelines (301 A-F, 302 A-C) define standard tests requiring positive reference substances to validate analytical system performance and bacterial consortium activity. However, historical controls have limitations: toxicological concerns (aniline, classified CMR), abiotic degradation biasing interpretation, or overly restrictive physicochemical profiles for diverse chemical substances.

Addressing these challenges, this study aimed to select and validate new positive controls meeting strict criteria: toxicological safety, solubility, low volatility, absence of abiotic degradation, and chemical relevance for OECD tests. Three substances were chosen from distinct chemical families to broaden representativeness: caffeine, adipic acid, and 1,5-pentanediol.

These substances were tested according to OECD protocols 301B/301F (ready biodegradability) and 302B (inherent biodegradability). Activated sludge from French wastewater treatment plants was standardized to reflect biological variability. Additional tests confirmed no abiotic degradation or inhibitory effects on bacterial activity.

Results show rapid, complete, and reproducible degradation for all three substances, systematically meeting OECD criteria (?60% in 14 days for 301 tests; ?70% in 14 days for 302 test). The absence of abiotic degradation independent of microbial activity and lack of toxic effects on the inoculum were confirmed, validating profile relevance.

These findings establish caffeine, adipic acid, and 1,5-pentanediol as reliable, safe, and robust positive references, offering a relevant alternative to historical controls. Their integration into biodegradability test guidelines would improve methodological robustness, inter-laboratory comparability, and environmental assessment quality in demanding regulatory contexts.

Furthermore, ongoing work aims to consolidate these results by confirming performance under OECD 301A conditions, strengthening cross-method reliability. Concurrently, this approach will extend to polymeric materials to assess its relevance for complex matrices and support future polymer environmental evaluation developments.

3.04.P-Tu164 Illuminating Persistence Assessment: Environmental Relevance of Light in OECD 309 Testing

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Standardized testing protocols for assessing aerobic degradation of chemical substances in surface waters typically involve incubation in continuous darkness for extended periods of time (60–90 days, OECD 309). While this approach is intended to prevent direct photolysis, it inadvertently suppresses key biological processes driven by light, particularly those involving photosynthetic microorganisms. Phototrophs possess diverse metabolisms and contribute substantially to biogeochemical cycling. Light exclusion likely reduces microbial consortia proliferation and may lead to significantly lower degradation rates. Furthermore, current test requests by ECHA allow only a minimal amount of suspended solids, limiting microbial diversity and activity, which potentially underestimates the true environmental degradation potential of substances.

This study investigates the role of non-UV photosynthetically active radiation (PAR, 400–700 nm)—with an intensity selected to represent light conditions typical of European surface waters—in supporting microbial biodegradation processes. Experimental results and literature analysis confirm that light exclusion reduces microbial growth and can lead to significantly lower degradation rates. In contrast, illumination with environmentally relevant non-UV PAR enhances microbial community function and can result in different degradation outcomes. In addition, oxygen released by phototrophs may help to maintain aerobic conditions in closed test vessels—an important approach in degradation testing of volatile substances.

Cyanobacteria and microalgae are integral to aquatic ecosystems and are among the most abundant organisms on Earth; recognizing their metabolic versatility and ecological significance is essential for advancing the robustness and environmental relevance of chemical persistence assessments. Consequently, test protocols that disregard the role of photosynthetically driven microbial interactions may not adequately reflect degradation under realistic environmental conditions.

We propose integrating controlled non-UV PAR into standardized test methods, especially pelagic tests, to better represent the ecological dynamics of surface waters. However, even with PAR light, the pelagic OECD 309 test may still fail to determine substance persistence reliably and reproducibly, underscoring the need for guideline revision.

3.04.P-Tu165 Investigating Closed Test Setups and Application Approaches for Biodegradation Testing of a Volatile and Hydrophobic Substance in Surface Water (OECD 309)

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Understanding the biodegradation of chemicals in the environment is an important element of hazard classification, exposure assessment, and persistence assessment. Standardised biodegradation simulation tests are available to assess degradation rates in different environmental settings. However, it is recognised that certain substances can be difficult to test due to their physico-chemical properties. Substances that are volatile and hydrophobic are recognised as especially problematic. The OECD 309 test (Aerobic Mineralisation in Surface Water) faces particular challenges due to the requirement to apply and maintain the test substance within an aqueous medium. Abiotic losses of the test substance can occur at various stages of testing, including test substance application, testing phase, sampling and analysis. Such losses affect the recovery of the test substance, impacting the mass balance and the reliability of results. Further, the partitioning of the substance out of the aqueous phase reduces the bioavailability of the substance and artificially lowers the observed degradation rate. Various efforts have been made to try to address these challenges, but the issues are not yet solved for OECD 309 guideline compliant testing.

In this study, various experimental approaches were investigated for testing the volatile and hydrophobic substance decalin (decahydronaphthalene) under OECD 309 test conditions. These approaches centred around testing decalin in a closed system, and investigating some of the approaches used in a previous OECD 309 test where silicone oil was used as an application solvent for dosing the test substance. Further, efforts have been made to develop more appropriate volatile traps, and alternative dosing methods based on passive dosing or water-miscible solvents. The results of these investigations will be presented, demonstrating the significant challenges with dosing, testing and recovery of the test substance. Experiments with silicone oil showed a high tendency for decalin to remain partitioned within the silicone oil, which carries implications for the bioavailability, degradation and volatilisation of the test item. Based on the results obtained under this study, decalin proves to be an extremely challenging substance to test under OECD 309.

3.04.P-Tu166 Comparison of OECD 301 & 310 Guideline Studies Regarding Their Capability to Degrade Substances with Limited Bioavailability

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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, predominantly water solubility and octanol-water partition coefficient (log K_{ow}). The statistical evaluation of respective publicly available data sets retrieved from the OECD eChemPortal demonstrated a significant correlation, implying that substances exhibiting poor bioavailability fail ready biodegradability tests with higher probability. A substance failing such a test is considered potentially persistent and may require the next level of testing, e.g. simulation testing, although the root cause for not reaching the pass level may be a test system that impairs bioavailability.

The data evaluation additionally revealed that studies according to test guidelines (TGs) OECD 301 & 310 differ significantly in their capability to degrade substances with limited bioavailability. Among other things, this suggests that TG OECD 301C causes less degradation of such substances than the other respirometric test systems. One explanation could be that the standard treatment of inoculum specific to the test system impairs its degradation potential.

Poorly soluble substances (water solubility <1 mg/L): only a (very) small share of the test substance is dissolved in the test medium and thus bioavailable for degradation. Studies following TGs OECD 301D, 301B and potentially OECD 310 (small sample size) appear best suited for degrading poorly soluble substances.

Partially soluble substances (water solubility 1-100 mg/L): test substances in this class are considered to be partially dissolved, the share depending on the guideline used. When neglecting the small sample size with TG OECD 310 data, the TGs OECD 301B, 301D and 301F exhibit comparable performance in demonstrating ready biodegradability.

Soluble substances (water solubility >100 mg/L): the test substance can be assumed to be completely dissolved. In this class, similar performance of all different guidelines studies is observed, which underpins the relevant role of bioavailability (substance completely dissolved) for good performance in biodegradation tests.

The results of this work suggest that it is worthwhile to aim for a well-devised study design to avoid over-conservative or false-negative results from biodegradability screening tests, including choice of the test guideline offering the best conditions for poorly bioavailable substances in practice.

3.04.P-Tu167 In-depth Understanding of Bacterial Communities in OECD 301 Biodegradability Tests : Towards Identifying Key Polymer Degradators

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Biodegradability tests, conducted according to Organisation for Economic Co-operation and Development guidelines (OECD), are essential for assessing the fate of chemicals on ecosystems. As biodegradability is primarily a biological process carried out by specific microbial communities, the in-depth characterization of bacterial populations in the inocula used in these tests has become fundamental. This is particularly relevant for polymer biodegradation, which relies on complex enzymatic pathways and specialized bacterial functions. To address this gap and better capture the biological mechanisms underlying polymer degradation, this study aims to i) identify the major bacterial degraders and their potential enzymatic pathways involved during biodegradability processes and ii) to deepen our understanding of polymer-degrading bacteria, in order to contribute to the development of more effective and predictive screening tests.

OECD 301F tests were performed using Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) micronized and non-micronized as well as Polyvinyl alcohol 8-88 (PVOH8-88).

Samples were taken at key time points: Day 0, D10, and D28 for controls (without polymer), and at D10 and D28 for test suspensions (inoculum + polymer). DNA was extracted and analyzed by high-throughput sequencing of the 16S rRNA gene, an environmental DNA technique allowing the identification of bacterial communities and their evolution during the biodegradability kinetics.

Preliminary results indicate that certain bacterial families show significant overexpression under polymer biodegradation conditions compared to controls. Taxonomic analysis, achieved with resolution down to the family level and in some cases to the genus and species levels, combined with biodegradation kinetics, reveals a clear association between polymer degradation and the selective activation of specific bacterial groups. Future work will extend this approach to additional polymers with varied chemical structures to identify a wider range of potential degraders and to refine the bacterial markers associated with biodegradation. In line with recent regulatory perspectives of European Chemicals Agency (ECHA, 2025), this work could ultimately support the development of faster and more relevant biodegradability screening tools, particularly for the assessment of polymers.

3.04.P-Tu168 Navigating the Unknown: The Critical Need for "Middle-Tier" Persistence Testing

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Persistence assessment has a vital role in evaluating long-term risks of chemicals in the environment and human health. Within different Regulations (i.e., REACH, BPR, CLP) persistence assessment and related information on degradation is needed for 1) PBT assessment, 2) hazard classification under CLP (PBT and vPvB, PMT and/or vPvM and aquatic environment) and 3) chemical safety assessment (e.g. exposure assessment).

The commonly accepted evaluation in a Tier 1 strategy is to initially use the results obtained from in silico tools and screening tests.

In silico screening tools, e.g. QSARs, can be used as part of a Weight-of-Evidence approach for the PBT/vPvB assessment, for instance predictions that the substance is not rapidly/readily degradable would support the conclusion that the substance is potentially P/vP.

There is a methodological gap between generic screening methods and complex higher-tier simulation tests.

Persistence assessment is based on either screening (relatively 'fast and easy') level ready/inherent biodegradability testing or 'complex and lengthy' higher-tier simulation type of testing. Current screening methods are stringent and often lead to a need of higher-tier testing when not conclusive. However, a negative result in a screening test does not necessarily mean that the substance will not be degraded under relevant conditions. Thus, 'middle' level testing methods which would address limitations of screening and higher-tier test methods are lacking.

The aim of this work is to demonstrate, through several relevant examples of biodegradability and persistence assessments, that the current testing strategy (in silico and screening) can lead to overly conservative conclusions regarding presumed persistence, whereas simulation test results demonstrate non-persistence. In this context, the conclusions of first-level tests can lead, in the context of industrial R&D, to a halt in development and may prove to be a barrier to innovation.

As part of the key areas of regulatory challenges addressed by ECHA in 2025, particularly regarding chemical pollution in the environment, it is necessary to develop and validate Middle-tier test that enable a more robust assessment of the persistence of chemical substances.

3.04.P-Tu169 Pre-Screening Biodegradability Potential of Chemicals Using an Artificial Bacterial Inoculum

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Biodegradability assessment is essential for evaluating the environmental fate of chemicals, but regulatory ready biodegradability tests are long, costly, and highly sensitive to the variability of natural microbial inocula. As a result, industries increasingly require robust pre-screening tools to identify chemicals with a high likelihood of success before initiating standard assays.

To support early decision-making, we developed a pre-screening approach based on a defined artificial inoculum composed of 44 bacterial strains representative of key functional groups in activated sludge. This controlled microbial system provides consistent activity suitable for early assessment of biodegradation potential.

Each strain was individually exposed to 30 chemicals with known biodegradability profiles, yielding 3,960 oxygen consumption assays over 28 days. A 5 % respiration threshold was applied to determine whether a strain responded to a given substance, and the proportion of active strains was used to classify chemicals into three biodegradability categories (BIN 1, BIN 2, BIN 3). Substances activating more than 27.9 % of strains were consistently associated with BIN 1, whereas those with fewer than 4.5 % active strains were assigned to BIN 3. Intermediate proportions reflected inherently biodegradable substances, although discrimination between BIN 2 and BIN 3 remained limited.

The artificial inoculum correctly identified 75 % of readily biodegradable chemicals reported in the literature and showed strong reproducibility. Importantly, no known non-biodegradable substance was misclassified as readily biodegradable. These results demonstrate that the method can rapidly flag chemicals with high biodegradation potential, reducing false negatives and guiding the need for further testing.

This approach is not intended to replace regulatory tests but to serve as a practical and reproducible pre-screening tool supporting industrial prioritization and early-stage environmental assessment.

3.04.P-Tu170 How to prioritise - interannual and interlake variability makes UV Filter risk assessment challenging

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As of November 2024, 47 UV filters are authorised for use in cosmetics within the EU, with common sunscreen formulations containing several in combination. These compounds can enter aquatic systems directly during bathing or indirectly via wastewater effluents. Current cosmetic authorisation does not require environmental risk assessment, and many UV filters have additional applications that may increase environmental inputs. UV filters have been detected globally in surface waters; however, Swiss data on lake exposure is scarce.

In 2024–2025, five Swiss lakes were monitored at multiple points in the bathing season, with one influenced by wastewater treatment plant (WWTP) discharges. Sampling covered surface layers, the water column, and sediments. Concentrations of 11 organic UV filters were determined using HPLC–MS/MS. Parallel controlled experiments in artificial ponds assessed the fate of UV filters from sunscreen use, with two ponds receiving sunscreen under WHO recommended application rates and two as controls.

Results showed variable distribution patterns across lakes and years, with octocrylene (OC) detected in almost all lakes at high concentrations, especially in surface waters. Other prevalent filters included 2-ethylhexyl salicylate (EHS) and homosalate (HMS). Some filters, such as 4-methylbenzylidene camphor (4-MBC), persisted in sediments despite being phased out years ago.

In artificial ponds, UV filters remained longer in surface waters than expected; OC showed extreme partitioning to the surface, while hydrophilic PMDSA was more evenly distributed. OC and EHS accumulated rapidly and persistently in sediments and biofilms, whereas PMDSA dissipated quickly. Six months after application, measurable OC and EHS persisted in water and sediment.

Overall, lipophilic UV filters are retained strongly in surface and benthic compartments, creating potential long-term exposure for aquatic life. Further work aims to establish sediment Environmental Quality Standards based on ecotoxicological testing to better protect aquatic ecosystems.

3.04.P-Tu171 What Are the True Surface Water Half-Lives of Readily Biodegradable Substances? A Case Study on Surfactants

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The half-lives of chemical substances in river water are important for assessing environmental persistence and estimating chemical concentrations through exposure modeling. Reports on the half-lives of readily biodegradable substances are limited, and regulatory guidelines provide default values for these substances in the absence of actual data. However, few studies have assessed the effect of experimentally derived half-lives on exposure estimates compared to default values. In this study, we investigated the half-lives of 12 readily biodegradable substances, including surfactants and tertiary amines, and evaluated the impact of the experimentally derived values on the accuracy of model estimation.

Most experimentally derived half-lives were less than 24 h, which is considerably shorter than the default values of 360 h (15 d) commonly used in regulatory assessment. When half-lives exceeded 60 h, biodegradability in surface water had a limited effect on concentration estimates in The National Institute of Advanced Industrial Science and Technology - Standardized Hydrology-based Assessment tool for chemical Exposure Load model, whereas half-lives below 24 h led to decreased concentration estimates. When concentration estimates using experimentally derived and default values were compared with environmental monitoring data, the experimentally derived half-lives improved the accuracy of the model estimations. These findings suggest that the experimentally derived half-lives of readily biodegradable substances considerably affect exposure estimates.

Our study suggests that readily biodegradable substances that show high exposure concentrations based on default values should be investigated further in higher-tier assessments with experimentally derived values, especially for surface water half-lives. In this presentation, the importance of experimentally derived half-lives and possible reconsideration of the default value will be discussed.

3.04.P-Tu172 Chemical Biodegradability and Persistence Assessment

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In consideration of ecotoxicological perspective regarding the impact of chemicals in the environment, indubitably it is important to ascertain the chemicals' persistence. Thus, a significant criterion is the rate of degradation in the environment and of particular value tests that can expeditiously screen out chemicals which are prone to degradation in diverse environments.

Biodegradation determination of chemicals is critical as it provides valuable insight of exposure and subsequently the long-term adverse effects on biota and implications for mankind [1].

Biodegradation screening tests (BSTs) have been developed and approved by the Organisation for Economic Co-operation and Development (OECD). BSTs are often a vital integral component of regulatory frameworks; OSPAR Agreement dealing with Harmonised Chemical Notification Format (HOCNF) is a casing point [2]. Its remit is the management of offshore exploration and production activities in the OSPAR maritime area. Their prominence in this context has been due to the associated relative low cost and presumed ease of implementation and interpretation.

However, biodegradation evaluation intrinsically is complex and unpredictable. Whereas the intended initial purpose of the BSTs was to screen out chemicals which are easily degraded, they have not evolved at the same rate as regulatory concerns, which now place an increased emphasis on environmental persistence.

Since the suitability of BSTs, for example OECD 306, is not deemed to be robust to identify persistent chemicals, projects to modify protocol have been instigated and proposal submitted to OECD for a new test guideline for a Marine biodegradation screening test for chemical Persistence assessment (MaP test). [3, 4, 5]. The MaP test incorporates increased bacterial cell numbers and uses manometric respirometers to run for 60 days or more. It has undergone ring-testing with the participation of 13 laboratories in 7 countries. [6] Does it have application in marine regulatory frameworks and conventions?

3.04.P-Tu173 Weight of Evidence Evaluation of Persistence for a typical UVCB Substance Using Persistence Assessment Tool (PAT)

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Assessing the environmental persistence of complex substances is critical for regulatory decision-making and risk assessment. A UVCB substance (unknown or variable composition, complex reaction products or biological materials) was evaluated for persistence using a weight of evidence (WoE) approach integrating several studies including: 1) ready and inherent biodegradation screening tests (OECD TG 301F, 302C) on the UVCB whole substance; 2) aerobic mineralisation in surface water simulation test (OECD TG 309) for the major constituent of the UVCB; 3) Quantitative structure–activity relationship (QSAR) modeling for 5 known constituents

covering approx. 90 % of the UVCB content; and lastly, QSAR modeling for 4 transformation products identified in the OECD 309 test.

Based on the available data, the result of the above studies was as follows: Screening tests for the UVCB indicated inherent biodegradability (73% in 28 days) but did not meet the criteria for ready biodegradability (50% in 28 days). Surface water simulation studies showed concentration-dependent degradation kinetics: at higher concentration (100 µg/L), the substance degraded rapidly ($DT_{50} = 37.5$ days), remaining below the regulatory persistence threshold, whereas at low concentration (10 µg/L) degradation was slower ($DT_{50} = 87.4$ days). This inconsistency could be due to variability in microbial community development across concentration treatments. QSAR predictions consistently indicated rapid biodegradability for all major constituents and transformation products. Due to the multitude of possible persistency interpretations for this complex substance, a solution was needed to enable a conclusive evaluation of all this data.

The Persistence Assessment Tool (PAT) was employed to quantitatively weigh all lines of evidence (LoE) and to enable transparent integration of these diverse data and LoEs, accounting for dataset quality, consistency, and strength of evidence. Despite some uncertainty from borderline results and concentration effects, the overall WoE based on expert judgement and PAT supports a conclusion of non-persistence for the UVCB substance. This case study highlights the value of quantitative WoE tools in environmental risk assessment, providing robust, reproducible conclusions for complex substances.

3.04.P-Tu174 The evaluation of the persistence of UVCBs in regulatory testing

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Evaluating the environmental fate UVCBs presents unique scientific and technical challenges in standardized biodegradation studies. CROs frequently rely on OECD Test Guidelines 307, 308 and 309 to generate regulatory-ready data. However, these guidelines were originally designed for single, well-defined chemical entities and therefore present inherent limitations when applied to multi-constituent and compositionally variable materials.

A primary challenge is characterizing test item composition and selecting appropriate analytes. UVCBs often contain multiple constituents, many without definitive structural identification, complicating the choice of marker components for monitoring transformation. This difficulty is compounded by limited analytical standards, variable ionization efficiencies, and co-eluting constituents, which collectively hinder quantification and the interpretation of mass balance.

Another major issue is representative dosing. Achieving environmentally relevant, homogeneous, and analytically tractable dosing is particularly difficult for hydrophobic, viscous, or surface-active UVCBs. Poor dispersion can lead to non-ideal exposure conditions, test system artifacts, and increased variability.

Additionally, sorption to soil, sediment, and test vessels often masks true transformation kinetics, complicating the calculability of DT_{50}/DT_{90} values and sometimes preventing kinetic modelling entirely.

Maintaining test system stability is further complicated by the fact that UVCB constituents may partition differently across phases challenging the assumption of a single, well-mixed compartment. These dynamics can blur the line between dissipation driven by transformation and dissipation driven by physical processes.

Radiolabelling, while a powerful tool for tracing parent and metabolites, is frequently infeasible because UVCBs lack a single representative structure amenable to labelling.

Together, these issues create significant uncertainty in regulatory fate assessments. This poster highlights typical CRO experiences, method adaptations, and emerging strategies—including targeted analytical profiling, fraction-based approaches, improved dosing techniques, and collaborative discussions with regulators—to improve study robustness while maintaining adherence to OECD requirements. Continued dialogue is needed to ensure that environmental fate testing frameworks evolve to accommodate the inherent complexity of UVCB substances.

3.04.P-Tu175 Assessing the Biodegradability of Water-Soluble Polymers: Scientific and Regulatory Perspectives for Safe and Sustainable by Design Innovation

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Water-soluble polymers (WSPs) are essential in home and personal care products. However, the complexity of these materials is not adequately addressed by current regulatory biodegradability tests, which were designed for simpler substances. This disconnect complicates the assessment of environmental persistence and the development of safe and sustainable polymer innovations. Current OECD and ISO biodegradability tests,

designed for low molecular weight chemicals, often underestimate WSP degradability due to high molecular weight, charge density, and limited bioavailability. This review identifies key limitations of existing test methods and proposes a mechanistically informed, tiered testing framework that aligns test selection with degradation pathways and environmental context.

The framework integrates polymer-specific adaptations to OECD/ISO methods, microbial profiling, and advanced analytical tools to improve realism and reproducibility. It also highlights the role of high-throughput screening for early-stage prioritisation and the potential of in silico modelling to accelerate biodegradable-by-design innovation. By combining empirical, mechanistic, and predictive approaches, the proposed strategy supports proportionate persistence assessments, enabling regulatory confidence and innovation aligned with Safe and Sustainable by Design principles.

Targeted actions for regulators, researchers, and industry are outlined to advance polymer-specific methods, data infrastructure, and predictive capabilities. The approach aims to enable more realistic, robust, and proportionate assessments of WSPs, supporting regulatory decision-making and sustainable innovation. Ultimately, this strategy will help ensure that future polymer development delivers both environmental responsibility and technological performance, in line with the latest scientific and regulatory expectations.

3.04.P-Tu176 Advancing Fate Assessment: Integrating Biodegradation and Microbial Toxicity Testing

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Understanding the fate of chemicals in the environment is essential for assessing exposure and risk. Current biodegradation screening tests, even when applied with modification, aim to demonstrate rapid and complete degradation, but some substances may not achieve these criteria. For such chemicals, particularly complex materials that may degrade slowly and form diverse intermediate metabolites, current frameworks only offer limited insight into transformation pathways or metabolite toxicity. When metabolites form, they may exhibit properties distinct from the parent chemical, creating uncertainty for persistence and risk assessment.

To improve understanding where standard tests and their modifications do not provide sufficient insight, we outline an alternative approach that combines established methods to address complex cases, within a tiered strategy including both in silico and laboratory-based experimental approaches. This approach integrates biodegradation screening, metabolite characterization, and microbial toxicity assays to provide a more complete picture of environmental fate.

The approach integrates four steps:

1. Extended biodegradation testing (OECD 301F) to determine degradation kinetics.
2. Identifying metabolites at multiple time points to characterize transformation pathways.
3. Assessing microbial toxicity of the test liquor using respiration (OECD 209) and nitrification inhibition (ISO 9509) tests, allowing comparison of metabolite toxicity with the parent chemical.
4. Evaluating the applicability and relevance of biodegradation data across environmental compartments, by testing in multiple matrices, including freshwater and activated sludge.

This combined strategy links chemical and microbiological perspectives, working alongside current frameworks toward a more comprehensive understanding of biodegradability in specific cases where regulatory or scientific needs require more detailed information than standard tests provide. Anticipated outcomes include improved understanding of degradation dynamics, metabolite formation, and associated toxicity risks. While broadly applicable to difficult-to-assess substances, its potential relevance for polymers and similarly complex materials is still being assessed. By integrating chemical and biological endpoints, this approach strengthens biodegradation assessment and offers insight under conditions more representative of environmental variability.

3.04.P-Tu177 Flow-Cytometric Quantification of Live and Dead Bacterial Cells in Environmental Inocula for Biodegradation Screening Tests

Kelly Jobling, Mathew Brown and Russell Davenport, Newcastle University, United Kingdom

Biodegradation screening tests (BSTs) such as ready biodegradability tests (RBTs) are key tools to evaluate and inform chemical persistence, environmental fate, and risk assessments. Standard protocols rely on low-density microbial inocula, often 100 – 10000 times lower than found in environmental matrices, and provide limited

characterisation of viable microbial cells – the primary drivers of biodegradation. The use of environmentally relevant increased microbial cell concentrations (ICCs) has been shown to reduce inocula variability and enhance test consistency, but the effect of their preparation on cell viability is unknown. Consequently, robust methods to quantify live microbial cells, in addition to total cells, are important in understanding their effect on biodegradation tests.

We have developed a flow-cytometric approach to enumerate live and dead bacterial cells in BST relevant inocula. Membrane integrity staining with SYTO 9 and Ethidium homodimer-2 was optimised for activated sludge and successfully applied to freshwater and marine samples, supporting cross-environment comparisons. Live/dead flow cytometry analysis is typically performed on fresh samples, but real-world BST workflows may limit immediate quantification. Therefore, we evaluated storage methods to determine whether samples could be preserved for accurate downstream analysis.

Activated sludge samples were stored at -80°C after preservation by two methods 1) glycerol and 2) glutaraldehyde fixation, and analysed by flow cytometry at 0, 7, 28 and 90 days. Glycerol preserved samples had significantly lower percentages of dead cells ($p < 0.001$) compared to fixed samples and did not show a significant increase dead cell percentage relative to untreated controls, indicating preservation of viability. In contrast, glutaraldehyde fixation showed elevated dead cell percentages compared to the untreated controls at later time points.

This flow-cytometry method provides a framework for live cell quantification in BST inocula and outlines evidence-based storage guidelines when immediate sample analysis is not feasible. Future work will compare this approach with complementary techniques such as 16S qPCR to further evaluate cell quantification methods. By improving microbial characterisation, this method supports reproducible and robust biodegradation testing, enabling correlations between microbial viability and test outcomes for environmental persistence and risk assessments.

3.04.P-Tu178 Colony Forming Units in Screening Tests: A Useful Predictor or Non-Informative Parameter?

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Colony Forming Units (CFU) are becoming increasingly important for characterizing inocula in OECD biodegradation tests, yet their predictive relevance remains uncertain. Furthermore a major limitation of CFU determinations in biodegradation testing is the lack of clear regulatory guidance on how the procedure should be performed. As CFU determinations are carried out after the start of the test, they cannot be used for pre test prediction. Since the result is only available during the test, the question arises as to whether the parameter offers any added value at all if the test meets the validity criteria. We compiled data from standard dilution plate counts from recent years and investigated whether there is a correlation between the performance of the inoculum and its CFU. We also examined whether it is possible to draw conclusions about the CFU based on dry matter of the inoculum, given that this is an important adjustable parameter within the framework of OECD 301. Although the CFU values depend on seasonal fluctuations as well as the operating conditions of the wastewater treatment plant, no relationship with degradation rates or test robustness was found. CFU results are highly method dependend and difficult to compare between laboratories or seasons. Furthermore, aerobic total variable cell count only detect microorganisms that are able to grow under the selected culture conditions, while many relevant organisms within the microbial community of the activated sludge remain uncultured. Overall CFU values represent a cultivation biased fraction of the microbial community present in the inoculum and therefore only provide contextual information but do not predict biodegradation outcomes.

3.04.P-Tu179 Validating Read-across Analogues Accounting for Metabolic Similarity. Application to Environmental Fate Endpoints

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Read-across is one of the most frequently used non-animal approaches for filling toxicological data gaps. Traditionally, analogues selection for read-across has been based on physicochemical, structural, and mechanistic similarity, while metabolic similarity has been largely overlooked. This could lead to inconsistencies, as structurally similar substances may exhibit conflicting properties due to differences in their metabolic fate. There is a universal realization that considerations of the metabolic fates of the target substance and proposed source analogue(s) are important in accepting or rejecting a read-across hypothesis. Assessing metabolic similarity is challenging because metabolic pathways are complex and influenced by both biotic and

abiotic factors. Comparisons of target and source metabolic information most often suffers from the lack of experimental data. These data voids have prompted the development and use of alternative methods. In silico tools that simulate (a)biotic transformations provide efficient instruments of assessing metabolic consistency when experimental toxicokinetic data are limited. Integrating these assessments into analogue selection can help explain differences in endpoint values among analogue chemicals and support the identification of more suitable source substances for read-across purposes. This will aid in establishing metabolic consistency between the chemicals used in a read-across argument (i.e., establish similarity in their (a)biotic transformations). Of particular importance are found to be the identification of common metabolic pathway(s), common metabolite(s), and similarity concerning the formation of reactive metabolites. Illustrative examples will be used to demonstrate the advantages of considering the metabolic information, provided by the OASIS software, showing that even closely related chemicals can differ in microbial metabolism, underscoring the need to include metabolic similarity assessment as a key criterion in regulatory decision-making. This work highlights the importance of incorporating metabolism information into read-across justifications to improve scientific confidence and transparency. The absence of such considerations remains a major source of uncertainty in many read-across arguments.

3.04.P-Tu180 New perspectives for AI-assisted prediction of biodegradability - a status report

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Models predicting biodegradability are useful tools to provide early-stage assessments that can serve different purposes:

- 1- For Regulatory professionals to prioritize chemicals in the absence of experimental data, mitigate concerns or flag up potentially persistent molecules with otherwise harmful properties such as toxicity
- 2- For Researchers to design environmentally friendly chemicals, i.e., help identify and prioritize candidates with expected favorable properties and provide alternatives of improved biodegradability, thus contributing to efforts to make chemicals safer for the environment and more sustainable.

As presented recently, both public and commercially available prediction models are currently unfit for purpose for design of biodegradable molecules. In addition, both from a scientific and regulatory point of view, they are in need of updates, given the fact that historic biodegradation data sets are characterized by a high occurrence of false negatives and that models using the same sets have been in widespread use for decades.

This study sums up developments with a focus on experimental data and model training sets, provides a critical assessment of the current situation and shows how significant improvements in prediction output can be reached.

Repercussions will be discussed for future developments of fit-for-purpose models targeted to regulatory or scientific audiences.

3.04.P-Tu181 Persistence Assessment of Azo-Dyes Based on Metabolomics Approach

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According to the REACH Regulation (EC) No 1907/2006, further information on degradation behaviour is required when screening-level biodegradation tests indicate potential persistence.

The persistence assessment must be conducted based on simulation biodegradation studies, such as OECD TG 309, which require radiolabelling or analytical techniques capable of monitoring the parent substance and its potential transformation products.

The structural complexity of azo dyes, together with their variable composition, makes radiolabelling technically unfeasible or economically prohibitive. It also limits classical analytical methodologies that, being targeted approaches, are unable to identify transformation products with unknown structure.

The goal of this project is the application of the E-fate Metabolomic Footprint (EMF) approach adapted to the traditional OECD TG 309, to investigate the biodegradation pathway of parent compound and its potential biotransformation products, with the proposal of their chemical structures.

Two kinetic studies, based on both OECD TG 309 and EMF, were conducted in parallel on the reference azo dye in natural surface water.

These complementary studies led to comparable kinetic values of the parent compound, confirming the

suitability of the metabolomics approach as an alternative strategy to assess the persistence properties of the reference dye.

In addition, the EMF study showed the kinetic pattern of by-products, confirming their complete degradation during the study and enabling the identification of a number of transformation products, with the proposal of the most relevant chemical structures.

In conclusion, the alternative metabolomics approach allowed the monitoring of the biodegradation behaviour of both parent compound and its transformation products, also enabling the proposal of chemical structures for the most relevant by-products. Overall, the EMF study can be considered a robust alternative approach for assessing the potential persistence of organic dyes, for which classical methodologies represent obstacles for the application of the standard OECD TG guideline.

3.04.P-Tu182 Determining Degradation Rates Using OECD 302B Methodology with Concurrent Size Exclusion Chromatography Analysis: Case Study with Modified Celluloses

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In previous studies, a method for analyzing molecular weight changes for various types of modified cellulose polymers over the course of a 302B using size exclusion chromatography (SEC) assessment was presented. Through this assessment, molecular weight and chemical alterations could be observed that would not be identified with the traditional OECD 302B measurements alone. This study aims to focus on a single cellulose type, hydroxyethyl cellulose (HEC) with varying molar substitution (MS) to determine the effect MS has on the percent degradation result, the molecular weight distribution, and degradation rate constant over the course of a 60-day OECD 302B assay. Six grades of HEC, with MS ranging from 0.5 – 1.7 MS, were tested in an OECD 302B. All samples tested were found to be classified as “primary, inherently biodegradable” following OECD classification guidelines. The three HEC grades with “low” MS were found to have a different pattern of molecular weight changes compared to those at “high” MS, such as a further decrease in molecular weight (MW) and narrower MW distribution. The lower MS materials were calculated to have a two-phase degradation rate constant of 0.5-1.66/day in phase 1, and 0/day in phase 2. The higher MS materials were calculated to have a two-phase or three-phase degradation rate constant of 0.36-0.42/day in phase 1, 0.02-0.03/day in phase 2, and 0.01/day in phase 3. This acts as a case study to assess the: (1) usage of concurrent SEC monitoring during an OECD biodegradation screening assay to be able to determine the differences small changes in physio-chemical properties (i.e., MS) have on the biodegradation profile, (2) ability to calculate degradation rate constants without using higher tier testing methods, and (3) identify potential transformation products for further toxicity/biodegradation evaluation.

3.04.P-Tu183 Environmental Emissions and Human Exposure to PBDEs in South Korea using Material Flow Analysis and Multimedia modeling

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Polybrominated diphenyl ethers (PBDEs), which are listed among persistent organic pollutants, have become ubiquitous in the environment and in human bodies due to their widespread industrial use as flame retardants. Human body burdens of PBDEs have increased exponentially, by approximately a factor of 100 from the 1970s to the 2000s. These lipophilic substances are known to enter the human body through various exposure routes, including environmental contact and fish consumption, and it is therefore necessary to quantitatively assess human exposure to evaluate their safety. In the present work, environmental emissions of four PBDE congeners (BDE-47, BDE-99, BDE-153, BDE-209) to air and water through their life cycles in South Korea were estimated using Material Flow Analysis (MFA). In addition, human daily intakes of PBDEs were simulated using environmental multimedia fate model K-SimpleBox and the European Union System for Evaluation of Substances (EUSES) exposure model. The estimated emissions of four congeners in South Korea indicated that atmospheric emissions were largely attributed to the production or product-use phase, depending on the substance. In contrast, emissions to water were predominantly driven by waste-treatment processes such as landfilling for all congeners. Annual human exposure through environmental and dietary intake showed distinct patterns by substance: low-brominated congeners were strongly influenced by water emissions, whereas highly brominated congeners such as BDE-209 were mainly driven by atmospheric emissions. The predicted human exposure levels were further compared with reference values to identify substances and life-cycle stages requiring improved exposure management. This study confirms that environmental emissions and human exposure to certain PBDE congeners may persist due to the waste-disposal phase of products, despite reductions in PBDE production and use following strengthened regulations in South Korea. However, as the modeling

results rely on several assumptions and involve considerable uncertainties, more refined follow-up studies will be required.

Disclaimer/Disclosure:

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3.04.P-Tu184 BiSX and BiSX/g-C₃N₄ (X = Cl, Br, I): Synthesis, Characterization, Photocatalysis of Crystal Violet Dye and Carbon Dioxide

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Global climate change and water pollution are two major environmental concerns intensified by rapid industrialization and the discharge of organic dyes, which are difficult to degrade by conventional methods. Photocatalysis offers an efficient and sustainable solution, converting light energy into chemical energy for pollutant removal and CO₂ reduction. In this study, BiSX (X = Cl, Br, I) photocatalysts and their composites with graphitic carbon nitride (g-C₃N₄) were successfully synthesized via a high-pressure hydrothermal method to enhance visible-light-driven photocatalytic performance. The BiSX/g-C₃N₄ heterojunctions were designed to facilitate charge separation and suppress electron-hole recombination.

The photocatalytic degradation of crystal violet (CV) dye and CO₂ photoreduction were systematically investigated under visible-light irradiation. Kinetic analysis based on the pseudo-first-order model revealed that the BiSbI/5 wt% g-C₃N₄ composite achieved the highest degradation rate constant ($k = 0.066 \text{ h}^{-1}$), which is 3.35 times that of pure BiSbI. Enhanced activity was attributed to improved band alignment, larger surface area, and efficient charge transfer between BiSX and g-C₃N₄. For CO₂ photoreduction, all BiSX/g-C₃N₄ composites exhibited superior CH₄ generation compared with pure BiSX and g-C₃N₄, confirming the beneficial effect of heterostructure formation. Among them, the BiSbI/75 wt% g-C₃N₄ composite demonstrated the highest methane yield ($0.5993 \mu\text{mol g}^{-1} \text{ h}^{-1}$) and selectivity (92.60%), indicating excellent visible-light response and CO₂ conversion capability.

Overall, the incorporation of g-C₃N₄ significantly enhanced the photocatalytic efficiency of BiSX by promoting charge separation, light absorption, and CO₂ adsorption. This work provides insight into the rational design of Bi-based heterojunction photocatalysts for dual applications in environmental purification and solar-driven carbon conversion, demonstrating the potential of BiSX/g-C₃N₄ composites as efficient and sustainable materials for photocatalytic systems.

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3.04.P-Tu185 Optimising the Adsorption System for Complex “Produced Water” Treatment: Exploring Mixtures of Sorbents with Variable Properties

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Crude oil extraction generates large volumes of wastewater known as Produced Water (PW), with quantities increasing significantly as wells mature. This PW contains hydrocarbons, heavy metals and dissolved salts, posing substantial environmental risks. Although physical, chemical and biological treatment processes exist, many advanced technologies are constrained by high energy consumption and operational costs. Adsorption remains a promising solution due to its simplicity, low cost, scalability and potential for regeneration. However, the use of single adsorbents limits treatment efficiency across diverse pollutant classes. This research addresses this gap by evaluating individual and composite adsorbent systems to enhance PW treatment performance.

Synthetic PW samples were prepared to enable controlled assessment of phenolic compounds and heavy metals in batch and column studies. Batch tests quantified pollutant removal and adsorption parameters for multiple adsorbents, including F400, RH550, RH700, softwood biochar and coconut shell, across defined particle size ranges. Initial experiments using 5 mg adsorbent per 50 mL sample showed that F400 (150–250 μm) effectively removed phenolic compounds, with preferential uptake following the order 2,4-dimethylphenol > m-cresol > phenol. Heavy metal adsorption followed the trend lead > zinc > manganese. Time-dependent distribution coefficients confirmed strong sorption of zinc, lead, m-cresol and 2,4-dimethylphenol, whereas phenol and

manganese exhibited weak affinity. Comparative assessments also highlighted differential interactions between pollutant classes and container materials.

A subsequent experiment using 10 mg of F400, RH550 and RH700 over four weeks reinforced these trends, particularly for heavy metals. An intraparticle diffusion model developed in MATLAB elucidated transport mechanisms within adsorbent pores, distinguishing internal diffusion control from film-based limitations. The combined experimental and modelling outcomes support the design of layered or mixed adsorbent configurations and contribute to a predictive column performance model. Overall, this work advances the development of efficient, low-cost adsorption-based filtration systems for sustainable PW treatment.

Disclaimer/Disclosure:

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3.04.P-Tu186 The Importance of Being Extracted – How the Choice of Extraction Methods impacts Persistence Assessment

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In simulation tests using soil (OECD 307) and sediment (OECD 308), the fate and behavior of chemicals belonging to different classes were assessed for regulatory purposes. Frequently, [14C]-labelled substances were used in these tests which allow to quantify non-extractable residues (NER). Total NER, i.e. the NER remaining in the matrix after exhaustive extraction, can be categorised in three NER types depending on their release potential or environmental relevance. Type I NER are defined as sequestered and strongly sorbed parent compound or its transformations products and have the highest potential for remobilization. Two methods, Silylation and EDTA extraction, make it possible to extract the type I NERs from the total NERs and to quantify their proportion.

According to ECHA guidance R.11 the choice of extraction procedure depends on its applicability and efficiency and should be evaluated & justified in a pre-study. One of the two recommended extraction procedures is an exhaustive Pressurized Liquid Extraction (PLE) with a standard solvent. Alternatively, the sample can also undergo a stepwise solvent extraction first, followed by PLE, both with a substance specific solvent. Compared to the stepwise solvent extraction, exhaustive extractions are conducted under harsher conditions (e.g. elevated temperature and/or elevated pressure) and thus release part of the molecules strongly sorbed to the matrix. Key endpoint in persistency assessment are degradation half-lives (DegT50). Since the update of ECHA R.11 and CLP guidance, total NER have to be considered for DegT50 calculation. Thus occurrence of NER effects persistence assessment of chemicals registered under REACH as well as biocides and affecting also pharmaceuticals and pesticides. As type I NER are of regulatory importance, identified amounts of type I NER after Silylation or EDTA extraction have to be considered in DegT50 calculations. In case neither Silylation nor EDTA extraction are feasible or were not performed, the total NER should be regarded as non-degraded parent substance and considered as such for DegT50 derivation. In this study we want to illustrate the importance of extraction methods applied to soil and sediment for determination of total NER from a regulatory point of view. Based on substance cases we discuss how the efficiency of the extraction procedures in pre-tests could be assessed and show their impact on the persistence assessment of industrial chemicals under REACH and CLP.

3.04.P-Tu187 Speciation of Non-extractable residues of pesticides in soil and sediment by 4-pool kinetic analysis

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This study explores the feasibility of speciation of non-extractable residues (NERs) of pesticides in soil and sediments by 4-pool kinetic analysis. The 4-pool refers to parent compound, metabolites, NERs, and CO₂. We developed the parent->NER, one-metabolite->NER, and two-metabolites->NER 4-pool models to capture the following characteristic kinetic behaviors of formation of NERs in soil and sediment respectively: (1) If a parent compound is bound as NERs, the metabolites (in the unit of % applied radioactivity) kinetically drive the evolution of CO₂ only; (2) if a metabolite (x) in a sequential (as opposed to branched) pathway is bound as NERs, the metabolites are split into two terms (i.e., m1(t) and m2(t)) which kinetically drive the formation of NERs and evolution of CO₂ respectively; (3) if two metabolites (x) and (y) are bound as NERs, the metabolites are also split into m1(t) and m2(t) but m1(t) kinetically drives the formation of NERs in a biphasic manner. By these three 4-pool models, we could identify the bound metabolites in soil and sediment for the seven pesticides

respectively. We find that a metabolite, even bound as NERs in soil, is not necessarily bound in sediment if it degrades faster in water than it diffuses/adsorbs onto the sediment or the binding is hindered under anaerobic conditions. However, if metabolites are bound in soil or sediment, we could identify bound compounds by 4-pool kinetic analysis. By including NERs as bound compounds, we can also improve kinetic modeling of pesticide degradation in soil and water/sediment systems respectively. This approach allows for assessment of environmental risk of NERs and evaluation of the persistence of a pesticide with NERs from a molecular level.

3.04.P-Tu188 Spatial Distribution of PCB 28 and PCB 153 in Deep Oceans Using a Global Multimedia Model

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Polychlorinated biphenyls (PCBs) are an important class of persistent organic pollutants, that are still widely detected in the environment despite a global ban. This persistence thus continues to be a threat to ocean health, human and environmental health by extension. While the behavior of PCBs in the atmosphere and surface ocean waters has been well studied, their fate and transport in the deep ocean is poorly known. Understanding the spatial distribution and transport of these pollutants in deep oceans is crucial for quantifying the role of oceans as the long-term reservoirs of PCBs. Additionally, it enables a better estimation of PCBs global residence times and their ability to be reintroduced into surface oceans, be exchanged with atmosphere, undergo long-range transport and bioaccumulate in marine food-chains. In this study, we expanded a widely used global multimedia fate and transport model, BETR-Global, to oceanic depths of up to 6000 m. Model simulations were run from 1930 to 2024 for two representative congeners PCB-28 and PCB-153. Modeled concentrations compared favorably with PCBs concentration in both surface and deep oceans. Our results showed that 82.5% of total oceanic PCB-28 exists in surface ocean, 13.1% between 200 to 1000 m and approximately 4.4% below 1000 m. In contrast, 13.9% of total oceanic PCB-153 exists in surface ocean, 15.0% between 200 to 1000 m and approximately 71.1% below 1000 m. Results thus showed a contrasting fate of PCB congeners, and that deep oceans will indeed form an important part of global PCBs cycle. Further detailed results will be presented during the conference.

3.04.P-Tu189 UV Filter Contamination in Indoor Dust across Residential and Educational Settings

Pablo Esteban, Irene Navarro, Covadonga Alonso, Silvia Royano, Juan Escobar Arnanz, Adrian De la Torre and Maria Angeles Martinez, Center for Energy, Environmental and Technological Research (CIEMAT), Spain

Organic ultraviolet (UV) filters are widely employed in industry as chemical additives in the manufacture of plastics, polymers, and pigments to prevent UV-induced photodegradation, thereby maintaining their original appearance, long-term stability, and physical integrity. Beyond their industrial use, UV filters are also extensively used in cosmetic and personal care products to protect the skin from ultraviolet radiation. Consequently, during the manufacture, use, and disposal of these materials and products, UV filters can be released and mobilized into different environmental compartments, where their presence can jeopardize the health of ecosystems. For this reason, this study conducted a quantitative assessment of 20 UV filters (industrial and cosmetic derivatives) present in dust from domestic and public indoor environments.

During 2022, a total of 84 indoor dust samples were collected from various locations across the Madrid metropolitan area, including urban residential settings and public spaces such as offices and primary and secondary schools. In the latter sites, both settled and airborne dust were sampled. Samples were processed using a QuEChERS procedure and analyzed by gas chromatography coupled with tandem mass spectrometry.

UV filters were quantified in all dust samples, with quantification frequencies (Qf) ranging from 57% to 100%. The pollution profile was dominated by octocrylene and 2-ethylhexyl p-methoxycinnamate (EHMC) (Qf = 100%) and two phenolic benzotriazoles, UV-328 and drometrizole (UV-P) (Qf = 99%). Interestingly, some compounds exhibited a wide concentration range, such as octocrylene (41,600 ng/g) and homosalate (HMS, 17613 ng/g), whereas others showed much narrower variability, with levels between N.D. 2 ng/g for UV-234 and N.D. 4 ng/g for UV-928.

The ubiquitous presence of UV filters in indoor residential and public dust samples highlights their continuous release from consumer products and persistence. As people spend most of their time indoors, the results underscore the importance of considering these settings as critical compartments for assessing human exposure to emerging contaminants and their potential environmental impact.

Disclaimer/Disclosure:

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3.04.P-Tu190 Occurrence of Tire Rubber-Derived Compounds in Sewage Sludge from Spanish Wastewater Treatment Plants

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Tire wear particles (TWPs) are continuously generated from road traffic involving the release of tire rubber-derived compounds (TRDCs) such as N,N'-substituted p-phenylenediamines benzothiazoles and benzotriazoles, among others. Wastewater treatment plants (WWTPs) can contribute to the diffusion of these pollutants into the environment. During wastewater treatment, many of these substances can be retained in sewage sludge, which is often applied to agricultural soils as fertilizer. This practice can facilitate their transfer to terrestrial ecosystems, where they may persist, bioaccumulate, and potentially enter food chains, therefore posing an emerging environmental concern.

This study investigates the presence of 25 selected TRDCs in 16 sewage sludge samples collected before and after the anaerobic digestion process from two WWTPs located in the Tagus River basin (Spain). Samples (1 g) were extracted using an optimized procedure based on sequential sonication (2x15 min) with acetonitrile and acetone/hexane (1:1), followed by centrifugation (3000 rpm, 5 min). Extracts were analyzed by UHPLC–MS/MS and the identification and quantification were carried out using isotopic dilution method. The methodology was validated (SANTE/2020/12830; SANTE/11312/2021), achieving good recoveries (71–117%) and repeatability (RSDr <20%) for all analytes.

TRDCs were present in all samples with total concentrations in the range of 183.9 and 2257.4 ng/g. High detection frequencies (Df >85%) were observed for 72% of the target compounds, confirming their widespread occurrence in sludge. The most abundant compounds were MBTH (179.3 ng/g, median), 2-OH-BTH (34.8 ng/g) and DBZA (19.1 ng/g). Interestingly, 6PPD-Q was frequently (100%, Df) found at variable levels (0.3–25.8 ng/g, min-max).

The monitoring of TRDCs in WWTPs contributes to broaden the knowledge of their environmental impact and associated risks in the ecosystems. These findings highlight the persistence of TRDCs in sludge even after treatment and suggest their potential release into amended agricultural soils, with implications for soil quality and trophic transfer.

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3.04.P-Tu191 Biodegradation of Tire Additives by Sludge and River Microbiomes: Impacts of Pretreatment, Seasonality, Co-Substrates, and Microbial Community Shifts

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Tire-wear particles (TWPs) contribute an estimated 3.4–5.9 million tonnes of particulate pollution to aquatic environments annually. These particles, beside the road matrix, are complex mixtures of rubber polymers, fillers, metals, and various tire additives (TAs), including antioxidants, vulcanization agents, and corrosion inhibitors. While many additives and their transformation products exhibit toxicity and/or persistence, quantitative data on their biodegradation remain limited. This study investigated the effects of (i) microbiome sources (activated sludge vs. river), (ii) inoculum pretreatment (washed/aerated vs. fresh), (iii) seasonality (winter vs. spring), and (iv) co-substrate (glucose) availability on the biodegradation of eleven representative TAs (6PPD, IPPD, diphenylguanidine, benzothiazoles, benzotriazoles, and HMMM) using the OECD 301F respirometry test. Microbial community composition and genetic material abundance were monitored through 16S rRNA gene sequencing and qPCR. Biodegradation was highest with fresh activated sludge, followed by

river microbiomes, and washed sludge. The effect of seasonal variation on biodegradation rates was more pronounced in river microbiomes, with compounds like hydroxybenzothiazole and diphenylguanidine degrading by over 70% in spring but less than 20% in winter. The addition of glucose as a co-substrate enhanced degradation of most TAs in sludge microbiomes but reduced it in river microbiomes. Chemical treatments induced shifts in microbial community composition over 28-days test period, with *Rhodococcus* dominating benzothiazole treatments, while Gammaproteobacteria, including *Pseudomonas* and *Pseudoxanthomonas*, enriching on IPPD and 6PPD. Among the tested additives, only hydroxybenzothiazole was readily biodegradable, while the others appeared more persistent, warranting further investigation to determine their half-lives. These findings indicate that microbiome profiling provides valuable mechanistic insight for biodegradation studies, revealing treatment-specific community shifts and identifying taxa potentially responsible for degradation, information not captured by chemical analysis alone. Given the large-scale global emissions of TWPs, these findings also have implications for persistence assessments under frameworks such as REACH, as well as for policy decisions regarding additive regulation.

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3.04.P-Tu192 Photooxidation of Polluting Oil Slicks: Linking Physical Changes and Chemical Transformations under Simulated Conditions

Manee Patanapongpibul, Anouk Volker, Wesley Browne and Nathalie Katsonis, University of Groningen, Netherlands

Catastrophic events such as tanker collisions and oil well blowouts, together with routine operational leaks from ships, ports, and industrial facilities, release oil that spreads across the ocean surface. These slicks now span an area twice the size of France. This pollution of seawater by oil slicks represents a significant environmental risk, affecting not only ecosystems but also economies and human health.

When exposed to sunlight, the oil slicks oxidize into persistent and potentially harmful products. The kinetics of this oil transformation under sunlight illumination remain incompletely understood. Our aim is to understand and predict the chemical transformation of polluting oil slicks, under solar illumination, in order to inform the development of safer and more effective oil spill response strategies, including optimized dispersant selection, dosage, and timing.

Our strategy involves an automated platform that simulates oil spill conditions to study photooxidation-driven degradation of light and heavy oils, linking physical and chemical changes. Oil film spreading is quantified over time, and associated changes in surface tension are measured to assess potential effects of photoproducts on oil-water interfacial properties. To explore environmental relevance, aqueous composition is varied in salinity and ion content to resemble marine and freshwater systems. Our early results show that both oil type and water chemistry strongly influence film thinning and photoproduct formation, allowing assessment of how water chemistry modulates degradation kinetics. This integrated approach connects physical behavior with chemical transformations under simulated spill conditions and lays the foundation for building a robust data set to support the predictive modeling of oil spill fate.

3.04.P-Tu193 Benzo[a]anthracene Triggers Metabolic Activation and Inflammatory Injury in Human Lung Cells via CYP450 Pathways

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Polycyclic aromatic hydrocarbons (PAHs) are pervasive air pollutants that enter the respiratory system through inhalation and undergo metabolic activation to elicit toxic effects. Although benzo[a]pyrene (BaP) is well characterized as a known human carcinogen, its structural analogue benzo[a]anthracene (BaA) remains comparatively understudied, particularly regarding respiratory toxicity. This study systematically compared the cytotoxic, inflammatory, and metabolic effects of BaA and BaP to address this gap. Two human lung cell models—BEAS-2B and A549—were used to assess differences in susceptibility and biological responses. Cells were exposed to BaA or BaP at concentrations ranging from 0.5 to 20 μ M for 12, 24, and 48 hours. Cell viability was assessed using the MTT assay, and interleukin-6 (IL-6) secretion was quantified via enzyme-linked immunosorbent assay. Furthermore, the activation of the aryl hydrocarbon receptor (AhR) signaling pathway and the expression of downstream enzymes (CYP1A1, CYP1A2, and CYP1B1) were analyzed by Western

blotting.

Both PAHs decreased cell viability in a clear dose- and time-dependent manner, with BaA consistently exerting stronger cytotoxicity than BaP and BEAS-2B cells showing greater sensitivity than A549 cells. Inflammatory responses differed markedly: BaP induced a rapid six-fold increase in IL-6 secretion in A549 cells, whereas BaA triggered only a moderate three-fold rise. Mechanistically, both compounds activated the AhR–CYP1 pathway but with cell-type specificity. A549 cells showed ligand-dependent AhR degradation, while BEAS-2B cells maintained stable AhR levels. CYP1A1 and CYP1A2 were strongly induced, whereas CYP1B1 showed weaker activation.

In conclusion, although BaA and BaP share similar metabolic activation pathways, they manifest distinct toxicological profiles: BaA acts as a more potent cytotoxic agent, whereas BaP serves as a stronger pro-inflammatory stimulus. These findings highlight BaA as a PAH of emerging concern and suggest that risk assessments relying solely on BaP equivalence may underestimate the specific cytotoxic hazards posed by other abundant PAHs in the atmosphere.

3.04.P-Tu194 ANALYSIS OF BILE AS A BIOMARKER OF ABIETIC ACID TOXICITY IN FISH

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Abietic acid is one of the acid resins produced by conifers such as *Pinus*. Considerable amounts of acid resins are produced and released in pulp and paper mill effluents and discharged into rivers and water bodies. In this study, through bile analysis, the possible deleterious effects of abietic acid were evaluated in fish (*Oreochromis niloticus*), under laboratory conditions, exposed to concentrations in the range of 150 to 180 µg/L. Fish were kept in groups of 10 individuals in 300L water tanks and fed with commercial feed. Bile samples were collected after a 10-day exposure period. The bile samples were extracted with different solvents and prepared for analysis by GC/MS-TOF, after derivatization with BSA or by methylation in a basic medium. Samples were also analyzed by fluorescence, monitoring emissions at 250 and 280 nm, with excitation at 356 and 458 nm. The obtained results demonstrated alterations in the bile acid profile, especially cholic and deoxycholic acids. Fluorescence analysis demonstrated significant alterations in the emission spectrum of the bile samples, indicating alterations in their composition.

Keywords: Resin acids, toxicity, bile, bile acids.

3.05 Bridging Science, Regulation, and Remediation on PFAS: Addressing Unknowns and Emerging Challenges

3.05.T-01 Exploring the Unknown PFAS Space in Time Series From the Rhine and Danube: A Multi-Laboratory Study

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Using sum parameters and screening approaches, recent studies have revealed that target analysis can considerably underestimate the environmental burden of per- and polyfluoroalkyl substances (PFAS). For suspended particulate matter (SPM) from the German Environmental Specimen Bank, the sum of PFAS concentrations determined by the direct total oxidizable precursor (dTOP) assay was up to 145 times higher as compared to target analysis for 41 PFAS. The ratio of the extractable organically bound fluorine (EOF) to the dTOP mass fraction increased after 2010, implying a higher proportion of organofluorine compounds not oxidizable to targeted perfluoroalkyl acids. To explore the delta, i.e. unknown precursors and other untargeted PFAS, and discuss time trends, seven laboratories investigated SPM extracts from the rivers Rhine (Koblenz, 2005-2021) and Danube (Jochenstein, 2009-2021). The covered chemical space was expanded by using both reversed-phase liquid chromatography and supercritical fluid chromatography coupled to high-resolution mass spectrometry. To evaluate the data, PFAS-specific suspect and non-target screening (NTS) approaches were applied.

Based on the in-house workflows of the participating laboratories, 617 prioritized features were reported, 24 by three or more laboratories. Relative peak areas and time trends agreed well with target analysis. As members of

a previously untargeted PFAS class, three keto-substituted fluorotelomer sulfonic acids (FTSA-ketones) were tentatively identified (6:2, 8:2 and 10:2), with the 6:2 homologue increasing over time. In addition, pentafluorosulfanyl (SF) PFOS was tentatively identified, potentially originating from aqueous film-forming foams. Similar to PFOS, SF5-PFOS showed a pronounced decline after 2009. FTSA-ketones may degrade to PFCAs in dTOP, adding to unknown precursors, while SF₅-PFOS has been shown to remain stable in dTOP but would still be captured in the EOF sum.

The results of this study demonstrate that a combination of targeted analysis, sum parameters, and suspect/non-target screening is essential to obtain a comprehensive understanding of environmental PFAS exposure.

Semiquantification will be conducted to assess how much of the delta can be explained and how much remains unresolved. In addition, selected raw datasets will be reprocessed to identify the major factors contributing to differences in reported features among laboratories and to compare the various platforms.

3.05.T-02 Defining the Use of Per- And Polyfluorinated Substances (PFAS) In Green Energy Technologies and Providing Information on Their Functions: The Case of Wind Turbines and Batteries

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PFASs are currently proposed for restriction under the EU's chemical legislation REACH (the universal PFAS restriction or uPFAS proposal). The aim is to address the risks these highly persistent chemicals pose to the environment and human health. Under the proposal, all uses, except those explicitly exempted, would be banned for manufacture, use or placing on the market as substances on their own. Detailed information on PFAS uses and functionalities is therefore crucial in making these decisions, as well as for other contexts including academia (e.g., alternatives assessments) and industry (e.g., development of new materials).

Prior work has catalogued PFAS uses across many sectors, but information on chemical function is often lacking. Here, we define both uses and functionalities in greater detail for the case of green energy technologies, specifically for wind turbines and batteries. To do so, we draw on the unique dataset of 5 600 comments from the uPFAS public consultation. We list candidate PFAS uses with various levels of information, starting from the chemical function, then the specific product or article component, and finally the broader area of use. By adopting the Functional Substitution framework we link the chemical, end-use, and service function of each listed candidate use. To verify a selection of these candidate uses, we assess the academic literature and engage directly with actors along the value chain for consultation and feedback.

In one prominent example, for wind turbines, we find that although some stakeholders indicated that wind turbine blades were coated with fluoropolymers, the verification step showed that other, more suitable, materials are actually used. Batteries, by contrast, are found across many areas of use, each with distinct technical requirements. Accordingly, the wide range of batteries on the market is included in our candidate list, incorporating available information on their specific functions. Drawing on additional sources, we identify which battery types typically require PFAS in certain components, and which components do not.

As a result, this work provides input for alternatives assessments and, in extension, for evaluation under the essential use concept. It also informs the universal PFAS restriction proposal and helps address the current data gaps noted for the energy sector.

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3.05.T-03 Estimated Costs and Impacts of Widespread PFAS Remediation Across Europe

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Regulations and public concern related to PFAS in drinking water, wastewater, and soils continues to evolve. However, PFAS remediation and treatment space to date lacks context about how much it would cost to remediate PFAS across a wide geographical extent and targeting multiple media. This study aims to estimate PFAS remediation costs and mass removal impacts for targeted environmental media across the 27 EU member states for two different use restriction scenarios based on publicly available data. Identified media with available data, high potential PFAS impacts, and perceived likelihood of future regulation included heavily contaminated soil sites, drinking water, landfill leachate, and wastewater effluent and sludges. While direct industrial

discharges to water and air are likely a major source of PFAS, those were not included here due to limited data availability.

Two scenarios were developed: a "legacy" scenario to reflect remediation of previously emitted long-chain PFAS and an "emerging" scenario to reflect remediation of ongoing emissions of PFAS, including short-chain and ultra-short chain PFAS like TFA, many of which are still used with increasing emission rates. Site data was sourced from publicly available datasets and verified through industry sources, journalists, and academic partners. Costs and PFAS removed were estimated based on commercially available treatment trains using either published cost curves with associated uncertainty metrics or Monte Carlo simulations based on available data. Achievable removal of PFAAs and precursors through remediation was estimated by multiplying mass or volume treated for each media by estimated concentrations for those PFAS from the literature.

Legacy estimates total about €1.1 to €6.0 billion/yr, while emerging costs (adding wastewater effluent/sludges and more intensive treatment to address ultrashort-chain PFAS) are €52 to €200 billion/ yr. Estimated achievable removal and destruction of PFAAs and precursors under the emerging scenario totals 1 ton/year to 20 tons/year. Compared to the estimated total EU annual use rates for PFAAs and precursors of 15,000 to 50,000 tons/year, the emerging scenario could address less than 0.5% of annual EU PFAAs uses. This reflects limited impact of remediating the targeted media for PFAS. Results demonstrate that environmental remediation of PFAS is not a sufficient, affordable, or plausible approach to managing environmental PFAS stocks.

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3.05.T-04 Advancing Our Understanding Point Sources of PFAS Emissions to Air

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Much effort has been focussed on understanding potential sources of per and polyfluorinated alkyl substances (PFAS) emissions directly to the ground and water environments. However there is increasing recognition that PFAS emissions to air are an ongoing and important source of environmental PFAS with potential for both local and global impacts.

In this presentation we consider some of the mechanisms by which PFAS can be emitted to air. We also discuss the activities, land uses and industries which may be resulting in point sources emissions of PFAS to air, and the gaps in our knowledge.

Research into aerial emissions from fluorochemical manufacturing sites illustrates the magnitude and wide area impact they have. However while industries such as semiconductor and textile manufacturing are recognised as being among the biggest global users of PFAS, there appears to be very little research into the magnitude of emissions from these activities.

It is well recognised that landfills have for many years been receivers of PFAS-containing waste, and household waste including PFAS will continue to be deposited to landfill even as regulations restricting new use of PFAS come into force. Concern is growing over the appropriate management of PFAS in leachate emissions from these sites, however the potential for aerial emission of PFAS should also be considered.

Pathways for emission include fugitive emissions and stack emissions. Stack emissions from many processes will be subject to abatement to control the emissions of other 'conventional' contaminants. There appears to be limited study of the effectiveness of these abatements for controlling the discharge of PFAS. Thermal treatments may alter and transform PFAS, but studies of incineration show that very high temperatures are required for complete PFAS mineralisation. It has been shown that products of incomplete combustion of commonly used PFAS (including those present in firefighting foams and fluoropolymers) can include Trifluoroacetic Acid (TFA) and other PFAS of concern within the atmosphere.

Further global effort is required to better understand these potential sources and their significance to future global management of PFAS. The authors will give recommendations on prioritisation of further work.

3.05.P Bridging Science, Regulation, and Remediation on PFAS: Addressing Unknowns and Emerging Challenges

3.05.P-Tu195 A curated PFAS polymer database: data extraction and chemical space mapping

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Per- and polyfluoroalkyl substances (PFAS) are widely used and highly persistent, yet most attention has focused on low-molecular-weight PFAS. Much less is known about PFAS polymers, even though they are used on a large scale and may act as both direct and indirect sources of non-polymeric PFAS. Their fuzzy naming, stochastic composition, and lack of standard structural identifiers mean they are poorly represented in public inventories and are rarely included in data analyses or modelling studies.

This work aims to systematically identify, curate and chemically characterise PFAS polymers from major public resources to support improved assessment of their chemical diversity and potential environmental fate. We developed a rule-based information extraction workflow using regular expressions for PFAS polymer extraction. Potential PFAS polymers were sampled and evaluated by experts, and overlapping entries between sources were removed. Polymer names were cleaned and deduplicated, and the resulting dataset was normalised for multivariate analysis.

The curated database contains more than 1,800 PFAS polymer entries with diverse naming conventions and structural descriptions, including relatively well-defined polymers and substances of Unknown or Variable composition, Complex reaction products or Biological materials (UVCB). A grey area was identified for polymers that meet the PFAS definition via perfluorinated end groups rather than backbone units, which are not clearly captured by current side-chain fluorinated polymer, perfluoropolyether and fluoropolymer classes. Ongoing work applies multivariate statistical methods to map chemical variation and explore classification schemes for PFAS polymers. The database and resulting analyses provide a new foundation for integrating PFAS polymers into chemical assessment, fate modelling, and regulatory discussions.

Disclaimer/Disclosure:

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3.05.P-Tu196 If Fluoropolymer Production is of Concern, What is its Future?

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As concerns over the ubiquitous global contamination by per- and polyfluoroalkyl substances (PFAS) and impacts on environmental and human health have risen, the regulatory response has initially mainly focused on non-polymeric PFAS. Production and use of fluoropolymers (FPs) has received more attention recently, with respect to their essentiality to modern societies and considerations of their life-cycle impacts. The European Union is grappling with this in their PFAS restriction process, which might restrict the use of PFAS in many consumer products, while allowing the production and use of FPs in industrial applications.

Here we review available evidence on life-cycle impacts of FP production, use and end-of life. Fluoropolymer production sites remain contaminated from PFAS used as polymer processing aids, also often called 'dispersion aids', which typically have been perfluoroalkylcarboxylic acids (PFCAs) such as PFOA and PFNA that have well-documented toxicities. In recent years, substitute fluoropolymer processing aids (such as hexafluoropropylene oxide-dimer acid, HFPO-DA) have raised similar persistence and toxicity concerns to legacy PFCAs used initially. In addition to processing aids, there is further evidence of major emissions of a wider range of PFAS, including monomers, oligomers, other reagents, and synthesis by-products during fluoropolymer production. The toxicity of many FP processing aids is well-documented, while the toxicity of many other PFAS emitted during production and fluoropolymers themselves is less established. Concerns remain over the persistence and disposal of fluoropolymers. The persistence of FPs coupled with their associated emissions during production makes it inherently difficult for regulatory authorities to protect the environment and human health from PFAS, while keeping FPs being produced for applications deemed essential.

Disclaimer/Disclosure:

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3.05.P-Tu197 Understanding Fluoropolymers and Their Life Cycle

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Per- and polyfluoroalkyl substances (PFASs) are a diverse class of synthetic chemicals that have attracted significant attention since the early 2000s. While extensive research and regulatory efforts have focused on non-polymeric PFASs, their polymeric counterparts remain comparatively understudied. This report, developed by the OECD Global PFAS Group, synthesizes scientific and technical information on the life cycle of fluoropolymers—a key subclass of polymeric PFASs characterized by perfluorinated methylene moieties (e.g., $-\text{CF}_2\text{CF}_2-$, $-\text{CF}_2\text{CH}_2-$) within the polymer backbone. The study begins with a comprehensive review of the chemical identities of fluoropolymers in the global market, including a non-exhaustive inventory of over 130 unique active Chemical Abstract Service (CAS) Registry Numbers. It then examines the full life cycle of these materials, covering production and use, the presence of other PFASs in commercial formulations, degradation during use and end-of-life processing, and environmental releases. Despite their widespread industrial and consumer applications, significant knowledge gaps persist—particularly regarding the presence of other PFASs in formulations, degradation pathways, and environmental emissions. Nevertheless, existing evidence shows that PFAS contaminants can originate from unreacted materials, intermediates, processing aids, byproducts, and degradation products throughout the fluoropolymer life cycle. Many of these substances are highly persistent and may remain in products or be released at various stages. This work underscores the need for comprehensive investigation into the structural diversity and life-cycle behavior of fluoropolymers while providing information to regulators and other stakeholders to improve risk management of fluoropolymers. The findings provide a foundation for global stakeholders to develop coordinated and effective strategies, while highlighting critical areas for future research and policy development. This report completes a series of three reports from the Global PFAS Group on polymeric PFAS.

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3.05.P-Tu198 Global Inventory of Fluoropolymer Production Plants and Their Associated Environmental Contamination of Per- And Polyfluoroalkyl Substances (PFASs)

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Fluoropolymers (FPs) are widely used across many sectors of industry and consumer products, due to their special properties including chemical, oxidative, and thermal resistance, and extreme durability. However, the life cycle of FPs, and especially their production, is associated with high emissions of a wide range of organofluorine substances including monomers, oligomers, reagents (such as processing aids), and reaction byproducts into the environment. Many of these are non-polymeric per- and polyfluoroalkyl substances (PFASs) that are highly mobile, persistent, and toxic. To begin to evaluate the worldwide environmental and health implications of FP production, a global overview of fluoropolymer production plants (FPPs) and their associated contamination is needed. In this study, we first identified, located, and documented FPPs worldwide, and then compiled previously published PFAS measurements in the environmental media surrounding them, including surface water, groundwater, air, dust, soil, sediment, plant material, human and animal blood serum and tissues.

We found 55 current and historical FPPs located across the United States, Europe, and Asia, in 44 different cities. Of these 45 locations, only 15 have associated PFAS measurements in environmental media reported in the peer-reviewed literature. PFAS concentrations were highest at or close to the FPPs (distance <1km) with concentrations in surface water reaching up to 100 000 µg/L and in soil or sediment up to 280 000 µg/kg dry weight. The measurements also show high concentrations in media at distances up to 150 km away from the FPP sites. Perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl ether carboxylic acids (PFECAs) were most often reported with very high concentrations, but many more PFASs (>230) were detected across all sites, highlighting the diversity of PFAS contamination. Finally, based on these data, we estimate that approximately 19 million people live within 10 km of an FPP (164 million in a 50-km radius, around 2% of the world population) and are therefore likely affected by associated PFAS contamination. This study shows the large global problem of FP production while also revealing gaps in measurement data, underscoring the need for increased monitoring, risk management, innovation in safe-and-sustainable-by-design FP alternatives, preventing non-essential FP uses, and comprehensive regulation covering all life-cycle stages of FPs.

3.05.P-Tu200 PFAS Emissions and Fragmentation Pathways During Fluorine-Based DRIE of GeSn Microstructures

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The semiconductor industry's shift towards sustainable manufacturing demands a better understanding of scope 1 process emissions, which arise primarily from plasma etching and other vacuum-based processes. Plasma etching is a critical step in device fabrication. This step enables high-precision patterns and surface cleaning through the use of reactive gas chemistries. However, these same processes can generate and release potent greenhouse gases (SF₆, SF_x⁺, CF₄, C₄F₈ and more), contributing to the industry's carbon footprint and limiting advances in sustainable process design and posing a significant challenge to the sector's overall sustainability efforts, which lower-emission become more important with increased demand for semiconductors. In this work, we investigate emissions produced during deep reactive ion etching (DRIE) of germanium–tin (GeSn) microstructures, a promising material platform for next-generation Group IV photonics. Etching experiments were conducted on an SPTS Rapier system using fluorine-based chemistries, including sulfur hexafluoride (SF₆), a persistent perfluoroalkyl substance (PFAS) with a global warming potential of approximately 23,500–24,300 times higher than CO₂. Gas-phase analysis was performed using a ToFwerk Process Analyzer equipped with an electron-impact (EI) ion source and a compact time-of-flight (CTOF) mass analyzer. Time-resolved mass spectra were correlated with process flow data to identify characteristic isotopic and fragmentation signatures of SF₆, including SF_x⁺ (x = 1–5) ions and other fluorinated species formed during plasma-induced decomposition and additional EI-induced fragmentation. In addition, fragment analysis revealed the presence of sputtered Ge and Sn species in the exhaust, indicating that the energetic fluorine plasma promotes both chemical etching and physical sputtering of the GeSn surface. These measurements provide detailed insight into PFAS fragmentation pathways, plasma–surface interactions, and the formation of secondary fluorinated by-products, contributing to provide primary data for the life cycle inventory of products manufactured through fluorine-based DRIE.

3.05.P-Tu201 Removal of Per- and Polyfluoroalkyl Substances (PFAS) From Water Using Superfine Powdered Activated Carbon in Laboratory Batch Tests

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Per- and polyfluoroalkyl substances (PFAS) present a major challenge for water treatment facilities and contaminated sites, where ensuring low concentrations in drinking water and preventing their release into the environment is critical. Increasingly stringent water quality regulations and rising treatment costs call for more efficient and sustainable remediation strategies. While granular activated carbon (GAC; typically, 0.4–2.4 mm) is widely used for PFAS adsorption, superfine powdered activated carbon (sPAC; ~0.001–0.003 mm), which is produced by wet-milling conventional powdered activated carbon (PAC; ~0.01–0.05 mm), offers substantially greater external surface area and reduced diffusion limitations, enabling faster adsorption kinetics and potentially more compact and cost-effective treatment. Although sPAC has shown superior performance for other micropollutants, its effectiveness for PFAS removal has not yet been systematically evaluated. In this study, several PAC materials were screened, and two were selected for detailed investigation using laboratory batch tests. Adsorption isotherms and kinetics were determined for both PAC and sPAC in groundwater, followed by evaluation of PFAS sorption behavior in different water matrices with varying dissolved organic carbon levels to assess competitive sorption effects. The influence of PFAS properties (e.g.

different head group, chain length) was examined to elucidate inter- and intra-class sorption trends. PFAS classes analyzed include e.g. perfluoroalkyl carboxylic and sulfonic acids, fluorinated ethers and zwitterionic PFAS. All PFAS analyses were carried out using liquid chromatography-high resolution mass spectrometry. These findings provide a basis for assessing the applicability of sPAC for PFAS remediation. A pilot-scale study is planned to validate laboratory results under realistic conditions. This work supports treatment strategies for PFAS-contaminated drinking water resources and discharge waters, contributing to the development of more efficient and sustainable mitigation approaches.

3.05.P-Tu202 PFAS sorption by sewage sludge biochar – influence of wastewater treatment line and pyrolysis residence time on sorbent performance

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Sewage sludge (SS) pyrolysis is a promising strategy to eliminate persistent contaminants such as PFAS while sequestering carbon and producing biochar (BC), a sorbent that improves soil fertility, water retention, and pollutant immobilisation. However, the influence of wastewater treatment processes (e.g., use of coagulants) and pyrolysis conditions (e.g., residence time) on sorption properties remains poorly understood, limiting scalable production and product quality.

This study compares porosity and PFOA sorption efficiency of sewage sludge biochars (SSBC) from two wastewater treatment lines: one conventional using metal-based coagulants and anaerobic digestion (ChemDigSSBC) and the other producing biologically treated, non-digested sludges (BioRawSSBC). SSBC from both lines were produced at residence times of 20, 40, and 50 minutes in a full-scale pyrolyzer.

ChemDigSSBC exhibited a surface area (115 m² g⁻¹) 13 times higher than BioRawSSBC (8.9 m² g⁻¹) in the pore size range accommodating PFOA molecules (> 1.5 nm). This difference was mainly attributed to the higher iron content in chemically treated sludges used for ChemDigSSBC (12 g kg⁻¹) compared to biologically treated sludges used for BioRawSSBC (2.8 g kg⁻¹). Residence time had minimal impact. Consequently, PFOA-sorption to ChemDigSSBC showed values for Freundlich adsorption coefficients (log K_F 5.00-5.07) two orders of magnitude higher than those for BioRawSSBC (log K_F 2.84-3.25). The difference in iron content in the final biochars (24 vs. 7.3 g kg⁻¹) likely also contributed to this effect, since iron oxides provide positively charged surfaces that bind PFAS head groups. Carbon content was not a key factor since ChemDigSSBC (35.3% C) outperformed BioRawSSBC (45.7% C).

Using literature data, we showed that biochars with iron content > 20 g kg⁻¹ exhibited high porosity (> 60% of pores > 1.5 nm), and strong sorption (log K_F values > 4.3) when pyrolyzed at > 700°C.

This study indicates that wastewater treatment processes can substantially influence PFAS sorption performance of biochar from pyrolyzed sludge, which is key if facilities aim to produce high-value sorbents.

3.05.P-Tu203 Exposure-Led and Preventive PFAS Regulation: Diverging Policy Models and Global Implications

Adel Malek¹, Jude Arokianathar² and Joanna Matthew², (1)Yordas Group, Canada, (2)Yordas Group, United Kingdom

Global regulation of per- and polyfluoroalkyl substances (PFAS) is developing rapidly, but through different policy models across jurisdictions. While the European Union has moved toward preventive, class-based regulation, the United States and many other regions continue to rely primarily on exposure-led and substance-specific controls.

This work examines the regulatory design of exposure-led PFAS governance outside the EU, as well as the factors that shape these approaches, including political structure, jurisdictional authority, and regulatory risk tolerance. The objective is to explain how evolving scientific information is translated into enforceable regulatory controls across fragmented systems, and how this contrasts with the more centralised European model.

The analysis applies regulatory intelligence methods, including longitudinal horizon scanning, comparative policy assessment, and review of federal, state, and selected international regulatory initiatives. Emphasis is placed on drinking water regulation, hazardous substance designation, discharge permitting, product-level restrictions, and reporting requirements, with attention to how these instruments distribute compliance and remediation responsibility.

Preliminary observations indicate that exposure-led systems are increasingly concentrating PFAS management and remediation obligations on site owners, utilities, and waste operators. At the same time, upstream production and use controls remain more limited. This structure can create periods of regulatory uncertainty for several sectors as standards evolve.

The findings highlight how differing regulatory models create uneven planning horizons, compliance burdens, and remediation responsibilities across the global PFAS value chain. These insights are relevant to researchers, consultants, industry stakeholders, and regulators seeking to manage PFAS risk under evolving and decentralised regulatory frameworks.

3.05.P-Tu204 From Substance-Specific Controls to Class-Based Regulation: The EU Policy Shift on PFAS
Adel Malek¹, Jude Arokianathar² and Joanna Matthew², (1)Yordas Group, Canada, (2)Yordas Group, United Kingdom

Per- and polyfluoroalkyl substances (PFAS) have historically been regulated in the European Union through substance-specific controls. More recently, this approach has evolved toward class-based regulation, reflecting concerns related to persistence, widespread occurrence, and long-term environmental management. This shift is now reflected in the proposed EU-wide PFAS restriction.

This work examines the policy and regulatory drivers behind the EU transition from targeted substance controls to a group-based risk management framework. The purpose is to explain how environmental considerations, substitution feasibility, and regulatory practicality collectively shaped the current direction of PFAS regulation, and how this differs from exposure-led approaches used in other jurisdictions.

The analysis is based on regulatory pathway mapping, horizon scanning of ongoing EU consultations, and sector-level use profiling across industrial, medical, and professional applications. Particular attention is given to fluoropolymers, semiconductor manufacturing, medical devices, and personal protective equipment, where essential-use considerations intersect with regulatory transition planning.

Preliminary observations indicate that the EU approach places strong emphasis on prevention, upstream control, and transition to alternatives, while many non-EU systems continue to focus primarily on downstream exposure management, such as drinking water standards and site remediation. At the same time, the pace of regulatory development has moved faster than the availability of harmonised technical guidance in several implementation areas, creating short-term uncertainty for regulated sectors.

This work shows how policy design itself is now being used as a primary risk management tool for PFAS in Europe, and how this approach is increasingly diverging from exposure-led control strategies elsewhere. The findings are directly relevant to regulators, remediation professionals, and industry stakeholders planning compliance, substitution, and long-term regulatory obligations under class-based PFAS controls.

3.05.P-Tu205 Watch your blindspot: How chemical regulations drive regrettable substitutions
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The need for an effective and dynamic chemical regulatory framework is increasingly evident. While chemicals contribute significantly to societal development and economic growth, hazardous chemicals pose major risks to human health and the environment. As chemical use increases, so does potential exposure, creating complex management challenges. Regulation is a key approach to mitigate these risks. For example, the EU's REACH Regulation seeks to protect health and the environment and promote substitution of the most harmful chemicals, while the Stockholm Convention targets persistent organic pollutants (POPs). In response, manufacturers have substituted chemicals such as PFAS, bisphenols, and flame retardants (e.g., PBDEs) with alternatives. However,

instances of regrettable substitutions where replacements are equally or more harmful are reported. This study examines how regulations like REACH and SC may unintentionally exacerbate regrettable substitutions, increasing risks to humans and ecosystems. We employed a mixed-method approach, combining survey (Likert-scale, multiple choice, free text), semi-structured interviews, and document analysis. Survey responses were analysed using descriptive statistics and qualitative data analysed using reflexive thematic analysis. 52 Participants completed the survey (Government: 13; Industry: 15; Academia: 7; Associations: 4; Consultancy: 10; NGOs: 2). We analysed 17 documents and conducted 31 interviews. When asked whether regulations exacerbate regrettable substitutions, 70% of government respondents, 50% from industry, and 13% from consultancy agreed (i.e., somewhat or strongly). Across all participants (N=43), about 50% agreed and 30% were neutral. Conversely, over three-quarters 95% (N=41) agreed that data availability strongly influences substitution outcomes, and 85% (N=34) viewed regulation as a major driver of substitution. Qualitative findings indicate that current frameworks may unintentionally promote regrettable substitutions due to prioritisation of market access for chemicals, fragmented regulatory approaches, exemptions (including data requirements), time-driven decisions, barriers to transparency and information exchange and the need to balance internal market functioning with chemical risk management. These findings highlight the importance of developing regulatory frameworks in a way that will ensure informed substitutions of hazardous chemicals such as PFAS.

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3.05.P-Tu206 Ensuring Safety in PFAS Replacement: Chemical Stability and New Product Formation in Plasma-Processed Bio-Based Coatings for Textiles

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Per- and polyfluoroalkyl substances (PFAS) are widely used for surface coating in textiles due to their low surface energy, which endows liquid repellent ability. However, growing evidence indicates that specific PFAS, such as perfluorooctanoic acid (PFOA), may be linked to human health adverse outcomes. In response, the European Union has implemented progressively stricter regulations on PFAS applications. Therefore, there is an increasing amount of studies on PFAS-free alternatives, e.g. cellulose, starch and chitosan. However, the safety of applying these PFAS-free alternative materials remains insufficiently understood.

This research, within the SSbD4CheM project, investigates the chemical changes occurring in bio-based coating materials for textiles during atmospheric plasma processing, a treatment that can potentially introduce oxidation and other chemical modifications to coating precursors. The tested bio-based formulations have already shown strong water-repellency on polyethylene terephthalate (PET) fabrics, with high water contact angles (>140°) and self-cleaning behavior against common liquids, such as coffee, milk, and red wine. To characterize the chemical changes and identify the transformation products, Liquid Chromatography–Mass Spectrometry (LC-MS) with different ionization sources, including Atmospheric Pressure Photoionization (APPI) and Electrospray Ionization (ESI) were applied, and reactions such as epoxidation have been observed. The toxicity of newly generated transformation products will be assessed through the SbD4Skin tool that incorporates quantitative structure-property relationship models for skin toxicity endpoints prediction. By integrating chemical characterization with toxicity prediction, this work will provide an evaluation of the overall safety of applying PFAS-free materials in textile coating applications.

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3.05.P-Tu207 Closing the PFAS Mass Balance - Multi-Approach Data Processing for Enhanced Workflows.

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Comprehensive non-targeted screening of PFAS is essential to help scientists with closing the mass balance of PFAS in the environment. An untargeted LC-MS method employing a sensitive, high-mass-accuracy quadrupole time-of-flight (QToF) instrument coupled with a novel data-processing approach was used to participate in the PFAS NTA Interlaboratory Study organized by the National Institute of Standards and Technology (NIST) in 2024.

Three unknown samples (A, B, and C) obtained from NIST were analyzed to identify PFAS following the guidelines for the 2024 NTA ILS. To ensure maximum coverage of the PFAS chemical space analyzed a data-dependent acquisition (DDA) method in both positive and negative ionization modes was implemented using QToF mass spectrometry. The DDA method m/z trigger settings were validated using a standard mix with targets from method EPA 1633.

Multi-approach data processing included identifying PFAS compounds using NIST Library Match, the NIST suspect list, FluoroMatch and LabSolutions. This systematic and complimentary approach allowed for accurate analysis and identification of PFAS in the unknown samples. The NIST MS2 library was converted into a software-compatible format to facilitate accurate spectrum matching. Identification through NIST Suspect Match relied on common product ions, neutral losses, and isotope Score, with a mass error threshold of less than 5 ppm. FluoroMatch analysis incorporated its built-in PFAS library and algorithms, utilizing Kendrick Mass Defect plots to identify PFAS and related families. Results were dereplicated, and PFAS were identified and scored according to NIST-defined criteria. For samples A, B, and C, there were 40, 57, and 105 negative PFAS matches, respectively, and 9, 44, and 29 positive matches.

In this presentation we will demonstrate the workflow and how the comprehensive multi-data approach benefits the PFAS identification.

3.05.P-Tu208 Transcriptomic Benchmark Dose–Derived Human Equivalent Doses Following Subacute and Subchronic PFOA Exposure in Mouse Liver

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Perfluorooctanoic acid (PFOA) is a persistent per- and polyfluoroalkyl substance (PFAS) widely detected in environmental and biological samples, raising concerns about potential health effects. The liver, a major site for xenobiotic metabolism and accumulation, remains insufficiently characterized regarding molecular responses to subacute and subchronic PFOA exposure prior to overt toxicity. In this study, benchmark dose modeling was applied to derive human equivalent doses (HEDs) for transcriptomic changes in mouse liver following 14-day (subacute) and 90-day (subchronic) exposure to PFOA via drinking water at doses 0.06, 1.5, and 22 mg/kg B.W./day, and 0.006, 0.06, and 0.6 mg/kg B.W./day, respectively. mRNA sequencing revealed a dose-dependent increase in differentially expressed genes (DEGs), with the highest dose yielding 3,470 DEGs in subacute study, and 1,898 DEGs in subchronic study. Analysis of transcriptomic HEDs yielded median and 5th percentile values of 572.4 and 31.7 µg/kg B.W./day in the subacute study, and 22.6 and 0.0036 µg/kg B.W./day in the subchronic study. Functional enrichment analysis identified fatty acid metabolism and xenobiotic metabolism as the most sensitive biological processes. Process-level HEDs indicated greater sensitivity of fatty acid metabolism compared to xenobiotic metabolism, with median HEDs of 96.8 and 167.5 µg/kg B.W./day in the subacute study, decreasing to 11.3 and 20.1 µg/kg B.W./day following subchronic exposure. The 5th percentile transcriptomic HED from the subchronic study fell below the estimated population exposure level of 0.45 µg/kg B.W./day, while process-level HEDs for fatty acid metabolism were within one order of magnitude of this exposure estimate. These findings suggest that current population exposure may be sufficient to change hepatic gene expression, including pathways involved in fatty acid and xenobiotic metabolism.

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3.06.P Chemical Additives in Consumer Products – Analytical Methods, Challenges, and Regulatory Needs

3.06.P-We204 Next Generation Chemical Risk Assessment of Plastic Additives in Consumer Products Using LC- and DART-timsTOF-MS

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Additives in plastic products, including flame retardants, plasticizers, and other substances, are intentionally added to improve product performance. Despite EU regulations on chemicals such as PBDEs, phthalates, and BPA, these chemicals still enter the market due to poor recycling practices or a lack of regulation in manufacturing countries. To ensure consumer safety by enforcing EU regulations, robust analytical high-throughput methods that determine the chemical composition of consumer products are needed.

This study assessed the extraction efficiencies of plastic additives, with a subsequent comparison of liquid chromatography-trapped ion mobility-mass spectrometry (LC-timsTOF-MS) analysis and high-throughput direct analysis in real time (DART)-timsTOF-MS workflow. The suspect screening lists used were based on relevant EU regulations the PlastChem database, and validated spectral libraries. Different approaches for extracting plastic additives were evaluated based on solvent extraction efficiency, compound coverage, and suitability for high-throughput workflows.

The best performing method was a two-step extraction using dichloromethane (DCM) and toluene. Re-extraction of the samples with methanol resulted in numerous features at RTs below 6 minutes, highlighting a gap of more polar compounds not extracted by DCM-toluene.

For the chemical analysis, TIMS was used to provide an additional separation dimension, which can enable the distinction of isomeric substances, such as certain phthalates. LC-timsTOF-MS analysis showed a variety of plastic additives, including antioxidants, biocides, and flame retardants. Initial experiments using DART-timsTOF-MS analysis with 12-QuickStrip sample cards have shown successful detection of plastic additives such as regulated phthalates, bisphenols and BFRs. The calibration curves for these compound groups showed good linearity, enabling quantification by DART-timsTOF-MS. Beyond this, DART offers several advantages: direct analysis without time-consuming dilution steps, the ability to analyse up to 12 samples within 2 minutes, and the elimination of large volumes of LC solvents. Full comparability assessment of LC vs. DART application is currently under assessment.

Altogether, these results highlight the potential of DART for high-throughput applications, making it particularly suitable for enforcement, where time-consuming and costly targeted methods need to be complemented by efficient suspect-screening approaches.

3.06.P-We205 Safe and environmentally sustainable textile revalorization via chemical oxidation

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Accounting for more than 60% of all textiles manufactured, clothing is the most extensive application.

Europeans are estimated to consume approximately 19 kilograms per person annually. However, still, less than 1% of worn-out apparel is recycled into new garments, thereby contributing to an increased environmental load via incineration or landfill disposal. A majority of all household textiles, approximately 7 million tonnes within the EU-27 countries, corresponding to 75%, were either incinerated or deposited in landfills in 2022. However, a new EU amendment concerning textile waste was introduced in 2025 to promote a circular economy for worn-out textiles, with collection systems to facilitate an appropriate recycling of textile waste. Here, we present a novel methodology for the revalorization of worn-out textiles as an alternative to incineration and landfilling, achieved through the oxidation of cellulose in cotton-based garments using 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO).

The analytical non-target screening, based on the pre-concentration of aqueous waste effluents and solvent extraction of cotton-based textiles, was achieved using liquid chromatography coupled to a high-resolution mass spectrometer with data-dependent acquisition. Data mining was performed using Compound Discoverer 3.3 for feature extraction, peak alignment and identification with a mass spectral library, mzCloud. Only candidates with an associated fragmentation spectra, within 1.5 to 27 minutes and a fold change criterion of at least ten times the blank signal were kept during data processing.

The screening resulted in approximately 1,900 statistically significant features, where 75% were found to be more significant in the cotton-based starting material, and 25% were more pronounced in the crude cellulose nanofiber (CNF) product, indicating a small degree of chemical transfer during the valorisation of cotton. Additionally, the oxidation process resulted in several molecular features that were more pronounced in the aqueous waste effluent compared to the starting material, indicating the release of transformation products. To evaluate the sustainability of the entire valorisation process, a toxicological risk prioritisation will be conducted based on the screening results. A thorough quantification of the chemicals present in the starting material, CNF, and aqueous waste effluents will be performed to determine their distribution and levels within the process.

Disclaimer/Disclosure:

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3.06.P-We206 Plastic Additives in Menstrual Products: from Analysis to Dermal Migration Experiments for Improved Exposure Estimates

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Menstrual products are fundamental to ensure menstrual health, which is recognized as a fundamental human right by the World Health Organization (WHO). However, some of these products have been shown to contain chemicals of concern for human health, including phthalates (PAEs). Menstrual products are used in direct contact with vulvar or vaginal tissues, which have a high chemical absorption capacity. Therefore dermal contact with menstrual products might be a relevant exposure pathway for plastic additives. However, information about the presence of plastic additives other than PAEs in menstrual products is still poorly investigated and it is still unclear if plastic additives can be released from these products to the skin. To fill this gap, we analyzed PAEs, organophosphate esters (OPEs) and alternative plasticizers (APs) in various types of menstrual products and performed dermal migration experiments using sweat, sebum and vaginal/menstrual fluid to estimate the amount of plastic additives that is transferred from these products to the skin. All types of menstrual products analyzed (sanitary pads, panty liners, tampons, reusable sanitary pads, menstrual underwear and menstrual cups) had detectable concentrations of PAEs, OPEs and APs. Across all types of menstrual products 7 PAEs, 15 OPEs, 8 APs were detected. 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 (median: 28856 ng/g; range: 5019–42193 ng/g) and OPEs (median: 1906 ng/g; range: 158–4068 ng/g), while the highest APs concentrations were observed in single-use sanitary pads (median: 8873 ng/g; range: 2830–11455 ng/g). Preliminary results from dermal migration experiments performed on single-use sanitary pads and panty liners showed that plastic additives can migrate from menstrual products and become available for dermal absorption. Measured easily releasable fractions (ERFs, i.e. the fraction of chemical that is released from a product and reaches the skin) ranged from 0 (<LOD) to 39% for OPEs, from 0 (<LOD) to 18% for PAEs and from 0 (<LOD) to 20% for APs. Experimentally derived ERFs constitute a step forward towards an accurate estimation of the contribution of the use of menstrual products to the overall exposure to plastic additives.

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3.06.P-We207 Virtual effect-directed analysis of granulated rubber: identification of bioactive chemicals

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Rubber is a ubiquitous material used in society. Chemicals are added to the rubber as processing aids, e.g. accelerators during vulcanization and to achieve desired properties, for example UV and ozone protection, leading to a complex chemical mixture in the final rubber product. Additionally, end-of-life rubber is frequently recycled into granulates used in playgrounds and artificial turf. This raises concern both for human exposure and leaching of poorly characterized chemicals, emphasizing the need for improved hazard evaluation and early-warning strategies.

In this study, we applied a virtual effect-directed analysis (vEDA) workflow integrating chemical profiling with bioreporter-based toxicological testing to characterize 21 rubber, rubber-like and alternative infill materials. Assays targeted key toxicological pathways relevant to endocrine disruption, genotoxicity and developmental toxicity.

Of the 21 samples, all but one activated the aryl-hydrocarbon receptor (AhR; 14–31,400 ng benzo[a]pyrene EQ/g), four activated the p53 pathway (0.04–0.53 µg actinomycin D EQ/g) and two activated the estrogen receptor α (ER α ; 530 and 1020 pg estradiol EQ/g). Targeted analysis of 87 polycyclic aromatic compounds (PAC) and GC-HRMS-based suspect screening were conducted.

Tentatively identified compounds were subset with bioreporter assay activity from databases and models, resulting in the tentative identification of 20 AhR- and 24 ER α - agonists and 13 potentially genotoxic compounds. Univariate analysis prioritized chemical features for quantification and toxicological confirmation. One P53-active compound, N-Isopropyl-N'-phenyl-p-phenylenediamine (IPPD) was confirmed and contributed approx. 50 % to the observed genotoxicity in the most p53 active sample. Phenylamine additives, while AhR active and detected in concentrations up to hundreds of $\mu\text{g/g}$, showed low potency, contributing little (<1 %) to the observed AhR activity. Methylated chrysene isomers conversely contributed substantially to the AhR activity. Notable differences in chemical hazards were observed between materials, with SBR showing the highest AhR and p53 activity as well as higher concentrations of quantified compounds compared to alternative materials.

Overall, this work demonstrates the capability of vEDA as an early-warning tool for detecting emerging hazards in rubber-based materials and highlights the need for continued assessment of recycled and alternative infill products.

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3.06.P-We208 Impact of Sample Pre-processing and Extraction method on Quantification of Chemicals of Interest in Plastics

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Plastic materials are complex mixtures containing numerous additives, non-intentionally added substances and impurities, yet information on the chemical composition of plastic products remains limited. This lack of transparency, combined with concern regarding impurities, underscores the need to characterize chemical levels in plastic products to ensure regulatory compliance and improve chemical safety.

Instrumental techniques for plastic chemical analysis, whether targeted or non-targeted, require sample processing and isolation of analytes. Most reported approaches rely on solvent-based extraction. However, sample preparation methods are not standardized, and no widely accepted protocols exist. This lack of harmonization limits comparability across studies and introduces bias due to large uncertainties in chemical extraction efficiency.

In this project we evaluated existing analytical methods for the determination of chemicals in plastics. Sample preparation approaches differ widely across studies. Solvent extraction can be applied to whole pieces or powders obtained after fine homogenization, often using cryogenic milling to prevent thermolabile compounds from degradation. The most commonly used technique is ultrasound-assisted extraction, often combined with additional agitation (vortex). The primary extract may require purification before instrumental analysis, typically employing filtration in syringe, sorbent clean-up, or precipitation of polymer with methanol when the polymer dissolves in the extraction solvent.

We investigated how polymer type, sample pre-processing, solvent choice, and purification technique of primary extract influence extraction efficiency. Nine polymers were studied, including polypropylene, polyethylene, polyethylene terephthalate, polyvinyl chloride, polycarbonate, acrylonitrile butadiene styrene, high-impact polystyrene, silicone, and latex. These factors were evaluated based on extraction efficiencies of plasticizers in each polymer type.

By integrating method practicality with extraction performance, this work aims to provide recommendations for plastic extraction protocols and emphasizes the need for harmonization to improve data comparability and transparency of chemical composition of plastic consumer products, ensuring better protection of human health and the environment.

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3.06.P-We209 Analysis of PFAS and Plastic Additives using DART-MS

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Currently, the widespread presence of contaminants such as per- and polyfluoroalkyl substances (PFAS), legacy contaminants and other persistent organic pollutants in consumer products has raised growing concerns due to their persistence, mobility, bioaccumulative nature, and potential health and environmental risks. The large number of different PFAS and their varied structures are a great obstacle for monitoring purposes. Nowadays, liquid chromatography tandem mass spectrometry (LC-MS/MS) remains the standard technique for the detection and quantification of PFAS. However, this requires extensive sample preparation, solvent use, and long run times, factors that hinder high-throughput screening in these diverse sample sets.

To tackle these challenges, Direct Analysis in Real Time mass spectrometry (DART-MS) presents a promising alternative. DART allows the ionisation of analytes from solid, liquid or gaseous samples under ambient conditions. In addition, the analysis of one sample can be achieved within seconds. However, the quantification of analytes in samples with more complex matrices can be inaccurate due to matrix effects and would require more tedious preparations. This study focuses on the optimisation of protocols for the analysis of PFAS and plastic additives in environmental samples and consumer products. The results of newly developed DART-MS protocols will be compared to traditional PFAS quantification methods, such as LC-MS/MS. Standard mixes of diverse PFAS and plastic additives (standard mix) are used for the optimisation of instrumental parameters and refinement of analytical settings. After the method is validated for performance and robustness, complex real-life samples will be analysed using the two techniques: DART-MS and an in-house routine LC-MS/MS method. We hypothesise that DART-MS can achieve comparable identification as LC-MS/MS with less analysis time. In conclusion, this study develops DART-MS protocols for reliable contaminant detection, ultimately supporting scalable environmental monitoring and public safety initiatives.

3.06.P-We210 Hazardous chemicals in common infant garments – occurrence, migration into sweat and washout effect from laundry

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Skin-close infant garments from the Swedish open market were investigated for hazardous chemicals. These are compounds that may migrate from the textile fibres, be absorbed by the skin, and cause skin sensitization or other health effects. Infants are considered especially susceptible due to their thin, not fully developed skin. In this study, we screened 60 garments for 50 target compounds known from our previous surveys to occur in clothing textiles using Automated thermal desorption combined with gas chromatography-mass spectrometry (ATD-GC/MS). The aim was to determine the occurrence frequencies and the concentration levels of these compounds, as well as to investigate the washout effect from laundry of selected garments. To evaluate the wash-out effect, 10 of the 60 garments containing the highest levels of chemicals were washed separately for one, five, and ten laundry cycles, and compared with the corresponding non-washed garments. Thirty compounds, including arylamines, quinolines, phthalates, benzothiazole, and nitrobenzenes, were detected in the 60 garment samples with detection frequencies up to 80% and concentrations as high as 1.4 mg/g. The laundry experiments showed that a substantial amount of most of the target chemicals remain in the polyester garments even after ten laundry cycles. The 100% cotton garments on the other hand were found to exhibit higher washout rates, probably due to interruption of hydrogen bonds between the chemicals and the cellulose fibre. This implies that chemicals in cotton may be more readily desorbed into sweat and lead to higher migration rates than chemicals present in polyester materials. When performing artificial sweat migration experiments, we found that chemicals in cotton showed a migration rate up to three times higher compared to polyester garments. The results indicate that light coloured cotton garments are more suitable for infants due to both lower occurrence frequencies and lower concentration levels, as well as a higher chemical washout effect of the investigated chemicals. It also indicates that washing new garments, especially cotton, may reduce the risk of both migration of and exposure to these hazardous chemicals.

3.06.P-We211 Presence of Phthalate Impurities in Sunscreens and Implications for Exposure

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The lack of compositional data for consumer products remains a widespread challenge for exposure assessment and pollutant source identification. Personal care products (PCPs) are one area where this information need is supported by regulatory requirements, with product ingredient lists identifying chemical components in a formulation. For many purposes, this provides sufficient information; however, it does not cover non-

intentionally added substances.

Based on recent concerns about the presence of di-n-hexyl phthalate (DnHexP) as an impurity from the production of the UV filter diethylamino hydroxybenzoyl hexyl benzoate (DHHB), we evaluated a range of sunscreen products on the Czech market for the presence of phthalate impurities. Products were selected based on ingredient lists, with a set of 27 products identified listing DHHB as an ingredient, and four sunscreens selected as controls that contained alternative UV filters. DnHexP was detected in 12 of 27 (44%) of the PCPs containing DHHB, and in none of the non-DHHB controls. The products containing DnHexP were diverse, including general and children's sunscreens, makeup, and lip balm. In products with detected presence of DnHexP, the concentrations ranged from 0.28 to 3.4 µg/g. Other phthalate esters, particularly shorter-chain phthalates that can be intentional additives in PCPs, were detected in five of 31 products (16%). Based on concentrations, prevalence and typical product use rates, we estimated exposure potential to European adults and children.

DnHexP is associated with reproductive toxicity and is restricted in the EU. The clear presence at levels exceeding 1 ppm in sunscreens and other cosmetics indicates a gap in information transfer requirements, as information on impurities in formulations is not systematically indexed.

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3.06.P-We212 Elevated Rubber-derived Compound Concentrations in Indoor Climbing Halls Cannot be Mitigated by Climbing Hall Design or Operational Practices, Emphasizing the Need for Material-Level Solutions

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Rock climbing shoe soles are a consumer product containing elevated concentrations of many rubber-derived compounds (RDCs), including those of toxicologic concern such as 6PPD and 6PPD-quinone. Friction between climbing shoes and rock climbing holds leads to continuous generation of fine rubber particles which become airborne and are inhaled by climbers and climbing hall employees. Hence, indoor climbing halls are a hotspot for rubber-derived compound exposure. The goal of this study was to broadly assess RDC contamination levels in climbing halls globally, and to relate variations in RDC levels to climbing hall design or operational practices (e.g., ventilation), in order to identify mitigation strategies. Volunteers collected samples from forty-one climbing halls across Europe and North America utilizing a dual-sampling strategy: 119 samples of foothold powder, representing the source of rubber particle generation, and 120 samples of settled dust, representing a composite sample of airborne particulate matter, were analyzed from all halls for fourteen RDCs. RDCs were detected in every sample analyzed, with concentrations ranging from several ng/g to several hundred µg/g. Unsupervised analyses (hierarchical clustering and principal component analysis) revealed distinct groupings of samples with similar RDC levels. These groupings were relevant on a climbing hall level, as most samples within a given hall clustered together. However, supervised analyses (redundancy analysis, partial least squares regression, and univariate correlations) revealed only very weak relationships between RDC levels and climbing hall characteristics. These results demonstrate that hall design and operation cannot effectively mitigate the problem of RDC exposure in climbing halls: mitigation will require material-level solutions, specifically safe and sustainable-by-design (SSbD) innovations in the rubber used in climbing shoe soles.

3.07 Coastal Environments: At the Intersection of Human Activities and the Open Ocean

3.07.T-01 Standardization of an Acute Toxicity Test Method for Corals: A Multi-Laboratory Prevalidation Study

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Coral reefs support immense biodiversity and provide critical ecosystem services, yet face unprecedented threats from anthropogenic stressors including chemical contamination. A fundamental challenge in coral ecotoxicology is the absence of standardized testing methods. No internationally recognized test guidelines exist for assessing toxicity in corals, leading to variable and sometimes contradictory results across different laboratories and study designs. This fragmentation undermines reliable environmental risk assessments. Standardized test methods—such as those established by OECD and ISO—have proven effective for other aquatic toxicology test methods by ensuring reproducibility and comparability across different laboratories and geographic locations.

To address this critical gap, we developed a 96-hour semi-static acute toxicity test method specifically designed for the coral species *M. digitata*, evaluating both sublethal (bleaching; EC50) and lethal (mortality; LC50) endpoints. To evaluate whether this method produces reproducible and comparable results across different laboratory settings, multi-laboratory prevalidation trials were conducted involving thirteen independent laboratories across six European countries (six in Germany, four in France, one in the Netherlands, one in Norway, and one in the UK), with each laboratory independently conducting the complete test protocol. The coordinated efforts of these partner laboratories have been fundamental to demonstrating the method's robustness and transferability. Results from the prevalidation trials demonstrate that estimated EC50 bleaching values cluster within a relatively narrow range, providing strong evidence that the coral toxicity test method can generate comparable results independent of laboratory location or operator. This consistency in bleaching response across geographically dispersed sites demonstrates the robustness and reliability of this endpoint for inter-laboratory standardization. This prevalidation study represents a significant milestone toward establishing an international ISO standard for coral acute toxicity testing, made possible through dedicated participation of each laboratory partner. By providing a validated, reproducible framework applicable across diverse laboratory settings, standardization of this method will enhance data comparability, strengthen hazard assessment protocols, and improve our capacity to identify the effects of chemicals on coral reef ecosystems.

3.07.T-02 Neurotoxic Effects of Metal-Rich Particulate Matter on Neotropical Native Fish Brain: Bioaccumulation and Enzymatic Responses

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Industrial activities in coastal areas release metal-rich settleable particulate matter (SePM) into the environment, raising significant concerns about its potential neurotoxic effects on aquatic life. Once in the water, SePM dissociates into metallic nanoparticles and becomes a source of metals/metalloids to biota, which bioaccumulate these contaminants and pose neurotoxic effects. This study investigated the biochemical and bioaccumulation responses in the brain tissue of the neotropical estuarine fish, *Centropomus parallelus*, after a 96-hour exposure to three concentrations of SePM (T1=0.01, T2=0.1, and T3=1.0 g L⁻¹) and a control group (CN).

Acetylcholinesterase (AChE) activity in the brain was measured as a biomarker of neurotoxicity, and 17 elements were quantified by ICP-MS. Principal Component Analysis (PCA) revealed distinct metal accumulation patterns among treatments, with the highest concentration (T3) strongly associated with Ce, Rb, Ni, and Zr. AChE activity remained unchanged at lower SePM levels but was significantly inhibited at the T3 concentration (1.0 g L⁻¹). This inhibition suggests a threshold beyond which neurochemical regulation is compromised. Spearman correlations identified a positive correlation with V ($\rho = 0.57$) and negative correlations with Rb ($\rho = -0.61$) and Ni ($\rho = -0.59$) on AChE activity. The findings indicate that SePM, depending on its composition and concentration, can induce early neurotoxic responses in estuarine fish, disrupt the cholinergic system, and potentially impair organismal fitness. AChE proved to be an effective biomarker for detecting sublethal effects from complex metal mixtures. These results highlight the urgent need for regulatory policies to control the release of particulate matter from industrial zones.

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3.07.T-03 In-depth PFAS Characterization in Benthic and Pelagic Estuarine Food Webs: Contamination Patterns and Trophic Transfers

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Over the past decade, emerging per- and polyfluoroalkyl substances (PFAS) have been increasingly reported in aquatic environments. Still, the environmental behavior of numerous of these emerging PFAS remain poorly understood. This study investigated trophic dynamics and contamination patterns across habitats of both legacy and emerging PFAS, in the food webs of common sole (benthic) and European sea bass (pelagic). Overall, 14 marine species, including plankton, polychaetes, shellfish, crustaceans and fish, were collected in the Seine estuary (France), a coastal ecosystem under high anthropogenic pressure. These samples were analyzed using a semi-quantitative suspect screening approach (LC-HRMS), supported by the use of an in-house suspect list of 866 compounds and AFFF as reference materials. A total of 93 PFAS belonging to 31 classes were detected, including frequently overlooked precursors such as X:2 FTAB and X:2 FTSA-HOPrS.

Some of them were detected at high concentrations, notably 6:2 FTAB, with concentrations up to five times higher (62 ng/g ww) than the highest PFOS concentration measured (12 ng/g ww). Contamination profiles appeared to be influenced by three main factors: the habitat, the trophic level and the metabolic capacities. A habitat influence was reported for the first time, with benthic organisms being mainly contaminated by long-head precursors (e.g., X:2 FTAB and X:2 FTSA-HOPrS), and pelagic organisms primarily by short head precursors (FASA and X:3 FTCA) and end products (PFSA and PFCA). Across food webs, concentrations of long-head precursors tended to decrease with the trophic level, while concentrations of end products and short-head precursors indicated biomagnification. This observation, also reported for the first time, suggested a biotransformation of long-head precursors at the highest trophic levels. Finally, species having a liver (fish) showed relatively lower levels of long-head precursors than invertebrates and crustaceans, strengthening this hypothesis of biotransformation processes. These new findings contribute to a better understanding of the environmental fate of PFAS in coastal ecosystems and call for the implementation of broader monitoring strategies and future studies on the biotransformation mechanisms of long-chain PFAS.

3.07.T-04 Land-to-Reef Pollution: Integrated Isotopic and Chemical Measurements for Ecological Impact Assessment

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Coral reefs are increasingly threatened by local stressors, particularly land-derived pollution (LDP) from excess nutrients, metals, and anthropogenic chemicals, a process often amplified by factors like rainfall and coastal development. A critical knowledge gap persists in quantifying LDP inputs and their impact on coral reef functioning, which hinders effective mitigation. To address this, the present PhD project performed an integrated approach on Curaçao's fringing reefs. This included (i) stable isotope analysis (SIA; $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and elemental concentration of Dictyota macroalgae for pollution tracing, supplementing this with (ii) SIA food web structure assessment, and (iii) using passive sampling with contaminant quantification and in vitro bioassays for chemical pollution monitoring and ecotoxicity assessment. The results confirmed that LDP is strongly affected by rainfall amplifying pollution runoff to nearby reefs. Macroalgal $\delta^{15}\text{N}$ values, indicating anthropogenic nitrogen input, positively correlated with elemental pollution (e.g. Cu, Pb) and coastal alteration. This anthropogenic nitrogen signal was tracked high into consumer food web at an impacted site, which also showed a compressed trophic niche and reduced carbon resource diversity. Furthermore, a wide range of anthropogenic chemicals (e.g. pharmaceutical and pesticides) were detected, with concentrations peaking in the wet season. Several of these compounds exceeded predicted no-effect concentrations, and in vitro bioassays showed responses approaching risk thresholds. The three lines of evidence confirmed that, i) land-based pollution is amplified by rainfall, ii) propagates through the food web, altering elemental and chemical concentrations, and iii) induces biological effects in coral reef ecosystems. Combined isotopic, elemental, and

ecotoxicity assessment provides a powerful framework for pollution monitoring, guiding scientifically supported mitigation and management efforts on coral reefs.

3.07.T-05 Derivation and application of effect-based trigger values for estrogen receptor-mediated activity in coastal sediments

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Target chemical analysis is widely used to monitor persistent toxic substances in the marine environment; however, it cannot capture contamination caused by unknown compounds. Effect-based monitoring (EBM) addresses this limitation by using in vitro bioassays to evaluate the biological activity of environmental samples, including the combined effects of known and unknown chemicals. Establishing effect-based trigger values (EBTs) is essential for implementing EBM, yet most EBT studies have focused on wastewater and surface waters, with limited attention to sediments. In this study, we derived sediment EBT values for estrogen receptor (ER)-mediated potency using three approaches (Options A–C) that incorporate relative potencies, no-effect concentrations, and mixture factors. Among the resulting thresholds, 0.062 ng E2-EQ g⁻¹ dw (Option B) was identified as the most appropriate, consistent with guideline-based EBTs for surface waters (0.009–0.049 ng E2-EQ g⁻¹ dw) and wastewater effluents (0.003–0.088 ng E2-EQ g⁻¹ dw). Applying the derived EBT to sediments from Lake Sihwa showed significant ER-mediated potency at all sites except M1 and M4. Silica gel fractionation revealed that the polar fraction (F3) contributed most strongly to ER activity. Within F3, only I2, I3, and I4 exceeded the EBT. RP-HPLC subfractionation further indicated that ER activity was concentrated in F3.3. Potency balance analysis showed that quantified target estrogens explained only 10–53% of total ER activity, suggesting substantial contributions from unidentified compounds. Nontarget screening of F3.3 from I3 and I4 identified 12 candidate ER agonists, predominantly pharmaceuticals. The six most abundant compounds were purchased for bioassay confirmation. Diethylstilbestrol (DES) and heptyl 4-hydroxybenzoate exhibited RePs of 1.5 and $1.6 \times 10^{??}$, respectively. DES accounted for ~40% of total ER activity in I3 and more than 100% in I4, indicating its importance as a major contributor. This study is the first to derive an ER EBT for sediments and apply it to evaluate sedimentary ER activity in Lake Sihwa. The results demonstrate that synthetic estrogens and pharmaceutical-derived contaminants can play a far greater role in sediment ER-mediated potency than previously recognized. These compounds should therefore be prioritized in future sediment monitoring programs and considered in management strategies to mitigate endocrine-disrupting risks in coastal ecosystems.

3.07.P Coastal Environments: At the Intersection of Human Activities and the Open Ocean

3.07.P-We213 Land Hermit Crabs as Coastal Ground Environmental Monitoring Partners

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Tropical and sub-tropical coastal ground level environment poses a challenge to aerial monitoring techniques due to the extensive vegetation coverage that creates large blind spots. Large study areas will require either an equally large array of ground level static sensors or a small group of roaming sensors. Despite the advancement in robotics, it remains cost-prohibitive to develop and operate robots to courier sensors up and down complex coastlines. They will also need to detect impending tropical storms and take shelter from surfs in order to avoid being swept out to the oceans. These specifications would invoke an astronomical amount of engineering. Here, we propose a much simpler solution by partnering with a native invertebrate: the land hermit crab. It is a pseudo-crab with that uses any appropriately sized receptacle that it can find to protect its soft abdomen, and gills (from drying out). It prefers the empty shells of turbo snails and other gastropods. When it outgrows the salvaged shell, it will look for a larger empty shell or take it from another hermit crab. Depending on species, its roaming range can be few meters from the coastline or as far as three hundred meters inland. However, it will always hug the coastline. It will also detect changes in air pressure and hides from storms in the nooks of rocks or trees. In this study, we demonstrated the possibility of partnering with the land hermit crab to courier sensors via a custom shell hugging harness. The sensor harness is shaped to conform to the spiral pattern of the gastropod shell and hence detachable. Unlike conventional animal tagging, this approach does not mandate the captivity. Instead, empty gastropod shells with sensor harness can be left on the coast and the land hermit crabs will simply pick them up during a shell swap. As a demonstration, we partnered with *Coenitobita brevimanus* to courier electronics and sensors such as solar-powered temperature and humidity sensor, and a salinity sensor (for salt water ingress monitoring) in an arena that simulate a coastal locality. We were able to remotely monitor temperature and humidity in the vicinity of the hermit crab. Furthermore, we also managed to map the border

between salt water and dry ground within the arena as the result of the hermit crab's movement. This demonstration of non-invasive partnership with local fauna for environmental monitoring will offer a sustainable alternative to robotics and drones-intensive approaches.

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3.07.P-We214 Spatial Variation of Mercury Concentrations in Grey Seal (*Halichoerus grypus*) Pup Hair Across the UK

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Mercury (Hg) is an environmental stressor of concern in marine ecosystems, and has known negative impacts on reproduction in many species. Lanugo, or natal hair, represents a significant portion of the total body burden of Hg in young pinnipeds and likely represents an important elimination pathway for mercury. Assessments of mercury contamination for seals at coastal breeding colonies around the United Kingdom (UK) were done over two decades ago suggesting low risk for wildlife; however, these were only from a few locations and in other high latitude coastal ecosystems, fine-scale regional variation in mercury has been observed. Here we aimed to quantify the contemporary total mercury concentrations [THg] in grey seal lanugo from across the UK. From 2012-2021, lanugo samples (n=149) were collected at colonies in Scotland and England that have shown divergent growth trajectories ranging from a decline in pup-production at colonies in the western isles, to exponential growth in pup production at mainland colonies along the coast of England in the North Sea. Median [THg] (ppm, µg/g dw) was higher at the three mainland English colonies (median: 5.8 ± 2.4 µg/g Blakeney Point; 7.4 ± 3.8 µg/g Donna Nook; and 9.4 ± 4.8 µg/g Horsey) than the Isle of May (median: 3.3 ± 2.8 µg/g) and Monach Isles (4.9 ± 2.7 µg/g) breeding colonies in Scotland. The maximum [THg] in this study was 17.7ppm, which is considered low risk for individual health. We will also present results identifying whether foraging patterns contribute to any regional or sub-regional variation, and will assess for changes in [THg] over time at sites with multiple years of sampling. This study provides valuable baselines for assessing the impacts of single or multiple stressors in marine wildlife experiencing rapidly changing coastal ecosystems.

Disclaimer/Disclosure:

This material is based upon work supported by the University of Alaska Anchorage ConocoPhillips Arctic Science & Engineering Endowment. Work involving animals in this study complied with all relevant ethical regulations and was licensed under the UK Home Office project 60/4009 or preceding versions and conformed to the UK Animals (Scientific Procedures) Act, 1986. Research was approved by the University of St Andrews Animal Welfare and Ethics Committee.

3.07.P-We215 Assessment of Multiple Stressors in a Mediterranean Coastal Lagoon, Through Toxicity Assays, Contaminants and Microbiome Analysis of Sediments.

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The combined effects of chemical pollution, eutrophication and climate change pose a significant risk to coastal lagoons. The Orbetello Lagoon (Tuscany, Italy) is subjected to multiple anthropogenic pressures, including urban and aquaculture discharges, legacy contaminants and emerging pollutants. This study investigates, through a multidisciplinary approach, how chemicals and physical stressors affect toxicity and microbiome composition of sediments, a crucial ecosystem component supporting the health and biodiversity of coastal lagoons. Sediment samples were collected in two different seasons (autumn and spring) at five stations.

Ecotoxicological assessments involved acute and chronic toxicity bioassays conducted at 20°C and 25°C on sediment elutriates (25%, 50%, 100%) using standardized methods with *Aliivibrio fischeri*, *Phaeodactylum tricornutum*, *Acartia tonsa* and *Ficopomatus enigmaticus*. Analysis of organic contaminants were conducted to evaluate the presence and distribution of plastic additives, including organophosphate esters (OPEs), phthalates esters (PAEs) and non-phthalate plasticizers (NPPs). The samples were processed using ultrasound-assisted extraction followed by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Environmental DNA (eDNA) analysis was performed using shotgun sequencing to characterize microbial biodiversity. The metagenomic analysis revealed 10 phyla and 83 species (4 Archaea and 79 Bacteria). Alpha diversity analysis highlighted differences between sampling sites, while beta diversity analysis showed different community compositions between the autumn and spring samples. Toxicity assays showed overall low toxicity, although significant effects emerged at higher elutriate concentrations at specific sites, with biological responses varying seasonally and being further influenced by the temperature of exposure. The integration of chemical, ecotoxicological and molecular data, performed through penalized linear regression, identified the relationships between contaminant concentrations (OPEs, NPPs and PAEs) and toxicity responses, and revealed how these interactions may be modulated by the bacterial communities. These data will provide a comprehensive understanding of lagoon sediments health status and how it is affected by multiple stressors, and supports the development of transferable tools for assessing and managing Mediterranean coastal environments affected by human pressures and climate change.

Disclaimer/Disclosure:

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3.07.P-We216 Contaminant Burden of Stranded Sentinels – Maternal Transfer of Legacy and Novel Persistent Organic Pollutants (POPs)

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Oceans cover the majority of the Earth's surface and are vital in oxygen production, carbon sequestration, resources, and coastal protection. However, anthropogenic activities have introduced significant pollutants, including persistent organic pollutants (POPs), into marine ecosystems. POPs are resistant to degradation and pose severe risks to wildlife and human health by disrupting endocrine, immune, and metabolic functions. Thus, even after regulatory bans, legacy POPs persist in the environment, while emerging contaminants like UV filters and musks raise concern due to their strong bioaccumulation potential.

High trophic level animals such as marine mammals are ecosystem sentinels, integrating the temporal and spatial scale of food webs through their life history. They are also particularly vulnerable to POP bioaccumulation due to their longevity and lipid rich tissues. These pollutants can be transferred in utero from mother to offspring, as well as postnatally through milk, threatening early development. Long-finned pilot whales (*Globicephala melas*), as oceanic dolphins occupying middle trophic positions, would not be expected to exhibit pronounced exposure to coastal pollutants. This study investigates whether legacy and novel persistent organic pollutants are nevertheless present and maternally transferred in mother-foetus pairs stranded on the Isle of Lewis, Scotland, in 2023.

Liver, muscle, and skin tissues were analysed for various groups of POPs, including polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), UV filters, and pesticides, addressing analytical challenges like matrix effects (i.e., the impact of matrix composition, sample preparation, and interfering compounds on the results) and recovery rates (i.e., the efficacy with which any given analyte can be extracted and quantified). Liver samples of all animals generally exhibited stronger matrix interference and overall higher contaminant accumulation, especially for lipophilic compounds. Foetus tissue samples contained a higher concentration of PAHs and selected UV-filters than then maternal matched tissue samples, as well as expressing different proportions of compound ratios. A less clear pattern was evident for PCBs and fertilisers. This highlights the importance of maternal exposure pathways in early-life pollutant accumulation, emphasising the need for further studies on the impacts of POPs on marine mammal health and their role in ocean ecosystem integrity.

3.07.P-We217 Testing environmental samples in an acute coral toxicity assay with adult *Montipora digitata* fragments

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Coral reefs are increasingly facing unprecedented pressure from a wide range of anthropogenic stressors, including (untreated) wastewaters, contaminated sediments, pesticides, heavy metals as well as home and personal care products. In combination with global stressors such as ocean warming, acidification, and habitat degradation, local pollution emerges as a frequently overlooked threat, emphasizing the need for robust, reliable, and environmentally relevant coral toxicity tests. In the context of method standardization under e.g. ISO, it is essential to demonstrate its applicability towards various environmental samples. In this study, we examined multiple types of environmental samples (e.g., sediments and seawater samples) collected from different coral reef sites exhibiting varying degrees of anthropogenic influence. Both whole samples and their chemical extracts were tested across a range of concentrations using a 96-hour acute coral toxicity test with adult *Montipora digitata* fragments. Different ecologically relevant endpoints, including mortality, tissue loss, and bleaching were measured to evaluate coral responses and identify both sublethal and lethal effects of environmental samples. Preliminary results revealed toxicity associated with sediments collected from polluted sites, demonstrating that these standardized tests can successfully identify harmful environmental conditions and discriminate between sites with differing contamination levels. These findings highlight the potential applicability of such approaches for routine monitoring and for integration into future standardized methods.

3.07.P-We218 Seasonal Variations of Heavy Metal Concentration in Commercially Important Marine Fishes, Shrimps and Crabs in the Bay of Bengal Coast and the Implications for Human Health Risk in Bangladesh

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Heavy metal contamination in marine ecosystems has become a growing environmental and public health concern in Bangladesh. Industrial discharge and coastal pollution contribute to the accumulation of toxic metals in seafood, threatening food safety and human well-being. This study was conducted to investigate the occurrence and seasonal variations of heavy metal concentrations in commercially important marine fishes, shrimps and crabs from the Cox's Bazar fishing zone of the Bay of Bengal coast as well as to assess the associated human health risks, augmenting food safety/quality and seafood export in Bangladesh. Therefore, commercial marine fish samples viz. koral or vetki (seabass, *Lates calcarifer*), Ilish (ilisha shad, *Tenualosa ilisha*), rupchanda (pomfret, *Pampus chinensis*), churi (ribbon fish, *Trichiurus lepturus*), Loittyta (Bombay duck, *Harpadon nehereus*), two shrimps bagda (tiger shrimp, *Penaeus monodon*; white shrimp, *Penaeus indicus*) and swimming crab (*Portunus pelagicus*) were collected seasonally (post-monsoon, pre-monsoon and monsoon) over a whole year from both fish landing centers and fishing boats. Concentrations of four heavy metals (i.e., Cd, Cr, Pb, and Zn) in edible muscle samples were analyzed by atomic absorption spectrophotometer (AAS), while the probabilistic human health risks associated with heavy metal-contaminated seafood consumption were assessed using a standard equation-based approach. The results showed that, among the tested metals, only Zn was detected in every sample (fish and crustaceans) over the seasons. In terms of overall metal accumulation, Zn content was found exceptionally high followed by Cr and Pb, whereas Cd was below the detection limit (0.001 mg/kg). Remarkably high Zn content in seafood of Bay of Bengal coast was possibly due to the interference of huge shrimp hatcheries activities. Relatively low metal concentrations were recorded in pre-monsoon (Zn: 9.32 to 20.3 mg/kg, Cr: <0.005 to 4.24 mg/kg, Pb: <0.01 mg/kg, Cd: <0.001 to 1.12 mg/kg) followed by monsoon (Zn: 15 to 50.2 mg/kg, Cr: 1.03 to 3.95 mg/kg, Pb: 0.17 to 50 mg/kg, Cd: 0.14 to 0.58 mg/kg) and post-monsoon (Zn: 6.17 to 128 mg/kg, Cr: 1.16 to 3.62 mg/kg, Pb: 1.01 to 1.53 mg/kg, Cd: 0.11 to 0.44 mg/kg). For Zn and Pd, the values of bio-concentration factor decreased in the sequence of crab > shrimp > fish, while the values showed descending order as shrimp > crab > fish for Cr and Cd. The results also exhibited significant ($p > 0.5$) variations in metals bioaccumulation in different seasons. The calculated hazard index (HI) for the general population (adults and children) was below the maximum limiting value (i.e., <1), indicating that the seafood of Bay of Bengal is safe for human consumption and possesses a great export potential. However, the study recommends periodic monitoring of toxic heavy metals in the commercially important seafood species to avoid any unexpected health hazards caused by the toxic heavy metals via seafood consumption. The findings of this study can be applied to improve seafood safety monitoring and environmental management in coastal Bangladesh. The data on seasonal and species-specific variations in heavy metal accumulation provide a scientific basis for establishing regulatory limits and updating national seafood quality standards.

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3.07.P-We219 Physiological and Ecotoxicological Effects of the Mineral Ultraviolet Filter Nano-Titanium Dioxide on Temperate Marine Microalgae

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Ultraviolet (UV) filters are active compounds found in personal-care products (PCPs), such as sunscreens and cosmetics, to mitigate photoaging and photocarcinogenesis by absorbing, blocking or scattering UV radiation. These filters, classified as either mineral-based (inorganic) or chemical-based (organic) are considered contaminants of emerging concern (CEC) due to their extensive global use and pervasive presence in aquatic environments. Key pathways into marine systems include direct skin wash-off, wastewater effluent and industrial discharge. UV filters have been detected globally across diverse aquatic matrices, including sediments, surface and offshore waters, and within aquatic organisms. While recent media and regulatory attention has focused on coral reef impacts – prompting bans in regions such as Hawaii and Palau – the effects on temperate marine ecosystems, particularly primary producers, remain underexplored. This study addresses this gap by investigating the physiological and ecotoxicological effects of nano titanium dioxide (nTiO₂), a widely used mineral UV filter often marketed as ‘reef-safe’, on the temperate marine microalga *Isochrysis galbana*. Using a multidisciplinary approach, microalgae were exposed to environmentally relevant concentrations of nTiO₂ under UV illumination for 96 hours. Responses were assessed via growth inhibition, chlorophyll-a depletion, changes in dimethylsulfoniopropionate (DMS) levels, oxidative stress biomarkers, enzymatic activity and ultrastructural changes via electron microscopy. Results show that concentrations between 500–1000 µg/L significantly impair growth and chl-a content, elevate oxidative stress markers (e.g. lipid peroxidation, protein carbonylation), and upregulate antioxidant enzymes such as catalase and superoxide dismutase. Even low concentrations (50 µg/L) caused measurable declines in growth and chl-a within 96 hours. These findings demonstrate that nTiO₂, even at low concentrations, can disrupt key physiological functions in marine microalgae, suggesting ecological risks to coastal primary producers. Despite growing recognition that nanoparticulate mineral UV filters may not be as environmentally benign as their bulk counterparts, nTiO₂ continues to be marketed as ‘reef-safe’ due to limited regulatory oversight. This study underscores the need for broader environmental risk assessments to evaluate UV filter impacts across diverse marine environments and organisms.

3.07.P-We220 Mediterranean Seafood Under Scrutiny: Chemical Burden and Regulatory Implications

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Persistent Organic Pollutants (POPs) are regulated under the Stockholm Convention due to their persistence, bioaccumulation, long-range transport, and adverse effects on ecosystems and human health. In parallel, contaminants of emerging concern (CECs) with similar properties remain largely unregulated, and occurrence data in wild seafood are still scarce. Current European legislation, including the Marine Strategy Framework Directive (MSFD), focuses primarily on legacy contaminants, potentially overlooking new or understudied substances. This study aimed to evaluate the occurrence of both POPs and CECs in Mediterranean seafood and to assess potential environmental risks and human dietary exposure, providing scientific evidence to support future regulatory prioritization.

A total of 91 samples from 13 wild-caught marine species were collected at six locations along the Catalan Mediterranean coast (Spain). Ten pollutant families were analysed, including organophosphate esters (OPEs), phthalates (PAEs), non-phthalate plasticizers (NPPs), bisphenols (BPs), UV-filters, musk fragrances, polybrominated diphenyl ethers (PBDEs), novel brominated flame retardants (NBFRs), dechloranes, and short-chain chlorinated paraffins (SCCPs). Compounds were quantified using LC-HRMS and GC-MS(/MS), depending on chemical class. Human dietary exposure was estimated through calculated Estimated Daily Intakes (EDIs), while environmental risk was evaluated using Risk Quotients (RQ = MEC/PNEC).

SCCPs dominated the total contaminant burden, showing the highest median concentration (750 ng/g wet weight), followed by PAEs and NPPs. Detection frequencies were highest for PBDEs (92.5%), OPEs (82%), SCCPs (80.7%), and PAEs (73%). Emerging substitutes for regulated flame retardants and plasticizers, such as OPEs and NPPs, were widely detected, highlighting shifts in chemical usage. Species- and site-specific differences were observed, with elevated NPP levels in European seabass and red mullet, and higher SCCP and NBFR concentrations at specific locations.

Estimated daily intakes were below established safety thresholds, but the co-occurrence of multiple POPs and CECs raises concerns regarding cumulative exposure. These findings emphasize the need to expand monitoring frameworks and consider additional contaminants, such as SCCPs, for inclusion in European seafood regulations.

Disclaimer/Disclosure:

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3.07.P-We221 Investigating The Effect of Coastal Pollution, Seasonality and Weather Conditions on The Coastal Air Composition.

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The ocean is home to a wide range of organisms, including bacteria, microalgae, seaweed, fish, and large marine mammals. Some of these organisms and the metabolites they produce can accumulate in the sea surface microlayer—a thin layer at the ocean's surface. When waves break, air gets trapped which creates underwater bubbles. These bubbles rise to the surface where they burst, picking up these metabolites and microorganisms that are then released as sea spray aerosols into the atmosphere.

Seasonal variations influence the chemical and biological makeup of coastal ecosystems, impacting air and water quality. In addition to natural processes, human activities like coastal industry and tourism can alter the composition of seawater and consequently also sea air.

This study aims to identify key environmental and biological factors shaping the composition of coastal sea air. We integrate datasets on physical parameters (wind speed, wave height, temperature), biological indicators (phytoplankton, bacteria, chlorophyll), and microorganisms found in sea air. Pollution metrics are also considered. Multivariate analyses are used to link environmental drivers to sea air composition.

We hypothesize that both natural variability and anthropogenic pressures significantly alter coastal air microbiomes and chemical profiles. Seasonal patterns and pollution indicators are expected to correlate with shifts in microbial diversity and sea air composition. Understanding these interactions is critical for assessing implications for environmental and human health within a one health approach.

3.07.P-We222 Beyond the Turbines: Exploring Sediment Effects of Offshore Wind Farms on Marine Benthos, *Corophium volutator* and *Hediste diversicolor*

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Offshore wind energy is central to decarbonization. However, its expansion may introduce novel chemical stressors to offshore marine ecosystems. Measures such as cathode protection galvanic anodes and anticorrosion paint used on offshore wind turbines may leach potentially toxic chemical compounds including trace metals such as cadmium (Cd), indium (In), lead (Pb), aluminum (Al), zinc (Zn), and gallium (Ga), and organic compounds such as bisphenol derivatives and per- and polyfluorinated substances, which may negatively affect marine organisms. Besides the well-known negative effects of offshore wind farms, such as accidental birds'

collisions, electromagnetic and sound pollution, little is known about the effects of chemical leaching from the supporting infrastructure. This study investigated the potential ecotoxicological effects of sediments collected from offshore wind farms on two key bioindicator species' i.e. marine ragworms, *Hediste diversicolor* (polychaete) and mud shrimp *Corophium volutator* (amphipod). The organisms were exposed to sediments collected from six sampling locations located within offshore wind farms and two reference locations in the Belgian part of the North Sea, outside the vicinity of the wind farms. Additionally, sediments collected at the test organisms' collection location (Eastern Scheldt, Netherlands) served as a control. Organisms were exposed to sieved (500 µm) sediments of each sampling location for 28 days in a laboratory setting, after which growth rate, survival, tissue metal accumulation, and, for *H. diversicolor*, energy reserves, including lipids, proteins, and glycogen concentrations, were measured. The study revealed no statistically significant negative impacts on *H. diversicolor* and *C. volutator* from exposure to sediment collected within offshore wind farms, with physiological responses comparable to locations outside the wind farms. However, negative trends suggesting declining growth rate and elevated tissue metal levels were observed in organisms exposed to the sediments from offshore wind farms. This research contributes to understanding the potential environmental impacts of offshore wind farms, particularly in terms of chemical pollution and that may impact marine ecosystems. Such insights are crucial for integrating ecotoxicological evidence into environmental risk management for sustainable offshore wind development.

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3.07.P-We223 Continuous Monitoring of Emerging Contaminants in Seawater and Oysters, Dún Laoghaire Harbour

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Contaminants of emerging concern (CECs) are defined as compounds that pose potential risks to ecosystems and human health but are not generally monitored or regulated. These compounds can be challenging to monitor due to their diverse sources, physico-chemical properties, and ubiquitous presence in the environment at trace levels. Biomonitoring is one method for investigating contaminants in marine systems. Marine bivalves have long been recognised as crucial ecological and bioindicator species within coastal ecosystems. Their wide distribution, sedentary nature, high filtration rates, and ability to bioaccumulate various contaminants make them exceptionally suitable for monitoring CECs. In Ireland, few studies on CECs focus on monitoring their levels in seawater or comparing biota with traditional grab sampling methods.

This work aims to continuously monitor seawater and oyster samples from Dún Laoghaire harbour in Ireland, following the deployment of over 18,000 oysters in the harbour in 2025. This is part of an oyster reef development project in collaboration with Green Ocean Foundation, seeking to develop and scale oyster reef systems in Ireland.

The contaminants examined include pharmaceuticals, pesticides, per- and polyfluoroalkyl substances (PFAS), nutrients, and metals.

Both seawater and biota samples were extracted using solid-phase extraction with Oasis HLB cartridges for analysis on an Agilent 1290 Infinity II LC system coupled to an Agilent 6470 Triple Quadrupole LC/MS. Quantification was performed using MassHunter software, and a matrix-matched calibration curve was generated to assess matrix effects. Along with target analysis, three cartridges underwent non-target analysis to ascertain any additional compounds in the samples.

Preliminary results from seawater samples collected before oyster deployment indicate the presence of pharmaceuticals and pesticides across four of seven sites studied. These include antibiotics, antimicrobials, ultraviolet filters, nonsteroidal anti-inflammatory drugs, herbicides, insecticides, and fungicides. Azithromycin, carbamazepine, clarithromycin, erythromycin, mefenamic acid, trimethoprim, and venlafaxine were confirmed at every site. Pesticides fluconazole and thiabendazole were also found to be ubiquitous across sites. This baseline provides important information to track trends in marine contaminants over time and to serve as a basis for studying oyster uptake and removal of marine contaminants.

3.07.P-We224 Land-to-Sea Subsurface Flow Transports Land-Derived Metal Pollutants into Coastal Coral Reefs

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Land-derived pollutants can enter coastal reefs through subsurface groundwater, creating hidden, chronic exposures that are often missed by standard seawater monitoring. As these pollutants move through sediment porewater, they can reach nearby organisms. This is especially concerning for coastal coral reef ecosystems, where local stressors like nutrient enrichment and land-based pollution can affect reef resilience alongside climate-driven bleaching. Understanding these local contributions is essential for effective reef management. This study applied a multidisciplinary approach to examine a fringing reef near a black sediment zone suspected of land-based groundwater pollution. The study combined three complementary investigations: (1) sediment porewater and seawater chemistry and metal concentrations, (2) elemental and trace metal accumulation in coral tissues and skeletons, and (3) reef ecological characteristics.

Results show no detectable chemical signals in sampled seawater, while porewater from the black sediment zone showed lower salinity, Mg, K, and pH, and elevated Mn, indicating a secondary water source entering the sediments. Analysis of organic tissues of the stony coral *Stylophora pistillata* revealed higher Mn and lower Fe and As in corals near the black sediment zone compared to the control site. No differences were observed in coral cover, bleaching, biodiversity, or community composition, suggesting that these chemical signals have not yet affected reef condition. Nevertheless, a recent bleaching event, together with previously undetected subsurface pollutant inputs, highlights the need for integrated approaches to detect chronic sublethal exposures before broader ecological impacts occur.

Elevated Mn in *Stylophora pistillata* tissues confirms that subsurface inputs reach nearby corals, showing that chronic, sublethal exposures can occur without immediate effects on reef community structure. This highlights the value of multidisciplinary approaches integrating porewater analysis, coral metal accumulation, and ecological surveys to detect hidden pollutant pathways and improve risk assessment in multi-stressor coastal reef environments.

3.07.P-We225 Heavy Metals and Rare Earth Elements in Sediments and Biota of the Southwestern Iberian Peninsula: Pollution and Risk Assessment

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Despite their importance, coastal ecosystems are under threat due to an increasing amount of pollution resulting from human activities. Heavy metals are a global concern due to their widespread distribution, inability to degrade, high toxicity at low doses and potential for bioaccumulation and biomagnification, depending on the metal, within the food chain. On the other hand, rare earth elements are essential for many technological products, and increasing their exploitation could pose an environmental risk in coastal areas. The aim of this study is to evaluate the presence and concentration of heavy metals and rare earth elements (REEs) in surface sediments and clams (*Scrobicularia plana*) along the south-west coast of the Iberian Peninsula. This area is subject to various sources of pollution and pressure, including industrial activities, mining, and urban development. Sediment and clam samples were collected in eight sampling points in March 2024. The surface sediment samples (3 cm surface layer, three replicates from each sampling point) were freeze-dried, sieved through a 63 µm mesh, and digested (0.5 g) using a mixture of 6 mL HNO₃, 2 mL HCl, 1 mL HF, and 6 mL H₃BO₃. The soft tissues of the clams (three replicates per sampling point, with 11 organisms pooled per replicate) were freeze-dried and then digested (0.3 g) with 4 mL HNO₃ and 1 mL HCl. All digestions were carried out using a microwave digestion system (Multiwave GoPlus, Anton Paar). Metal concentrations were determined using ICP-MS (iCAP Q, Thermo Fisher Scientific). Quantification of heavy metals and rare earth elements (REEs) in sediments, together with the use of estimated pollution indices, allows the identification of the most important pollutants and the sites with the highest levels of contamination. As, Zn, Pb and Cd are the major pollutants, with Rio Piedra and Palmones being the most impacted sites. According to the pollution degree recorded in aquatic sediments, *S. plana* clams accumulated both heavy metals and REEs, making this organism an important indicator of marine pollution.

Disclaimer/Disclosure:

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3.07.P-We226 Effects Of Gadolinium Exposure On Oxidative Stress In Two Brittle Star Species From The Ligurian Coast Of Italy

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Gadolinium-based compounds are widely used in medical imaging and are increasingly being detected in aquatic environments, raising concerns about their potential impact on marine organisms. Brittle stars are key benthic species that contribute to sediment turnover, nutrient recycling, and food web dynamics. However, their sensitivity to emerging contaminants, such as gadolinium, is not well understood. Although levels of gadolinium in aquatic environments have increased over the past decade, information on its physiological effects on aquatic organisms, including benthic echinoderms, is limited. This study therefore aims to evaluate the survival and oxidative stress responses of two brittle star species from the Ligurian Coast in Italy following short-term exposure to environmentally relevant and elevated concentrations of gadolinium.

A total of 270 specimens of one species and 180 of the other were collected along the Ligurian coast and kept in a controlled laboratory environment. The specimens were exposed to eight concentrations of Gd₂O₃ (i.e. 0, 1, 10, 50, 100, 250, 500 and 750 micrograms per litre) for seven days. At the end of the exposure period, specimens from each treatment group were sampled to assess potential sublethal physiological disruptions by analysing oxidative stress markers.

The analysis revealed concentration-dependent differences in oxidative stress profiles. Survival patterns showed no significant mortality across treatments. These results provide the first evaluation of gadolinium toxicity in brittle stars from the Mediterranean Sea. Furthermore, this study will contribute to our understanding of how emerging contaminants may affect the health of benthic invertebrates.

3.07.P-We227 Characterization of Emerging Contaminants in Coastal Sediments from Andalusia Using an LC-HRMS Suspect-Screening Workflow

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Contaminants of emerging concern (CECs) comprise a wide variety of compounds that differ in origin and chemical composition. Depending on their mobility and persistence, these substances can enter the aquatic environment and reach rivers and their interconnected ecosystems, including the coastal areas where rivers discharge. Liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) has expanded the possibilities for CEC monitoring in recent years through the use of suspect-screening techniques.

In this study, we aimed to detect and identify CECs present in sediments from the coastal areas of eight rivers subjected to different sources of pollution in the southwestern Andalusian region. Sediment samples were extracted using an ultrasound-assisted method with different organic solvents. A suspect-screening workflow was then applied using the NORMAN Digital Sample Freezing Platform and mzCloud as the main spectral library.

The results revealed the presence of 13 CECs—including pesticides, industrial chemicals, and other compounds—with high identification confidence. A semi-quantification approach based on compound ionization efficiency was subsequently applied to estimate ecotoxicological risk. Most CECs were detected at concentrations of a few ng/g, although some samples showed substantially higher levels of certain substances (e.g., DEET reached 25 µg/g at one sampling site).

These findings highlight the value of LC-HRMS tools for characterizing potential contamination sources in

environmental matrices and guiding future efforts toward the identified pollutants and to establish the potential risk assessment.

Disclaimer/Disclosure:

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3.07.P-We228 Suspect screening of contaminants of emerging concern in aquatic biota from the Andalusian Atlantic coast.

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The rapid expansion of industrial activities and the introduction of new chemicals have led to the occurrence of a wide range of contaminants of emerging concern (CECs) in the environment. These compounds vary greatly in origin and structure, and for many of them, ecological risks and potential health effects remain largely unknown.

This study investigates the presence of CECs in coastal aquatic biota (the bivalve *Scrobicularia plana*) from the Andalusian Atlantic coast using a suspect screening approach. The aim was to detect and semi-quantify a broad spectrum of pollutants, including pharmaceuticals, pesticides and industrial chemicals, that may pose risks to marine ecosystems but are not routinely monitored.

Field samples were collected from eight coastal sites in southern Spain during two campaigns (winter and summer). Sample extraction was performed using an adapted version of the “QuEChUP” protocol, which combines elements of Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) and Quick Polar Pesticides (QuPPE) procedures. Analyses were conducted by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Compound identification relied on a suspect screening workflow supported by the NORMAN Digital Sample Freezing Platform (DSFP) and the mzCloud spectral database.

Method validation showed good recoveries for moderately polar analytes, while very polar or highly hydrophobic compounds presented analytical limitations. In biota, six compounds were confirmed and semi-quantified. Five of them were naturally occurring metabolites (4-hydroxyphenylpyruvic acid, 4-hydroxybenzaldehyde, methyl picolinate, nicotinic acid and phenylpyruvic acid), reflecting endogenous metabolic activity and minor inputs from cosmetic or food-related sources. DEET was the only clearly anthropogenic contaminant, detected between <LOD and 47 ng/g, with the highest concentration at the Guadalquivir Bajo (GS) site, suggesting localized exposure to human-derived inputs and spatial variability in bioaccumulation patterns.

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3.07.P-We229 Human Activity in a Coastal Urban Area: Occurrence of Pesticides, Pharmaceuticals, and Sunscreen Agents in Dublin Bay

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Coastal environments near major cities experience continuous exposure to chemical pollutants from multiple origins: agricultural, wastewater effluents, runoff, and recreational use. Dublin Bay is a suitable site to explore the consequences of anthropogenic activity in a coastal system. The purpose of this study was to characterise the occurrence, distribution, and partitioning between water and sediment samples of selected pesticides, pharmaceuticals, and sunscreen agents, to evaluate how their presence reflects human activity in the area. These

contaminants of emerging concern (CECs) are monitored less frequently; therefore, information on their occurrence is limited.

Surface water and sediment samples were collected from eight locations around Dublin Bay. Water samples were extracted using solid phase extraction (SPE), while sediment samples were extracted using solvent based methods followed by SPE. Target analysis was carried out using liquid chromatography coupled with a triple quadrupole mass spectrometer. Analytical performance was assessed through recoveries, matrix effects, and detection limits relevant to marine testing.

A wide range of contaminants were detected with clear geographical patterns linked to human activity in their respective area. Pesticides (MCPA, mecoprop, thiachloprid) were more abundant in northern sampling sites, linked to the agricultural activity within the area. Pharmaceuticals (carbamazepine, clarithromycin, venlafaxine) and sunscreen agents (benzotriazole and sulisobenzone) were detected at sites associated with wastewater discharge and recreational activity. Repeated detection of contaminants across various sites highlights the complex nature of the occurrence and distribution of these chemicals in the coastal environment. The CECs detected are in the ng/L range, typically below regulatory levels. However, their presence as mixtures poses a threat to the marine environment.

These findings provide a chemical profile of Dublin Bay and show how combining water and sediment analysis can lead to understanding distinct features of human activity in the coastal environment. These results can go on to influence monitoring strategies in transitional and coastal waters. Further testing could influence the classification of priority hazardous substances monitored in the Water Framework Directive, or EU watch lists, where contaminants will be subject to Union-wide monitoring to assess the risks posed to the marine environment.

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3.07.P-We230 Flame Retardants in Dust from the Indoor Environments of Expedition Cruise Ships

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Flame retardants (FRs) are widely used in indoor environments to meet fire safety requirements. One understudied environment with respect to indoor chemical exposure to FRs is the maritime environment, particularly the indoor environments of cruise ships. With the continuous growth of the cruise industry, such environments with high chemical burdens can present a concern both for human exposure and environmental releases. This study presents the first comprehensive assessment of FRs in indoor dust collected from three expedition cruise ships of varying ages and refitting histories. Ten polybrominated diphenyl ethers (PBDEs), 23 alternative halogenated flame retardants, and 16 organophosphate esters (OPEs) were analyzed in dust from 12–16 locations per ship. OPEs, especially tris(1-chloro-2-propyl) phosphate (TCIPP), dominated the chemical profile, with concentrations reaching up to 1786 µg/g. Concentrations of FRs in different areas on the same ships differed greatly, sometimes by an order of magnitude. Older ships (built 2002/3) exhibited significantly higher FR levels compared to the newer vessel (Built 2020). Estimated daily intake modeling indicated that ship crew members, particularly those working in heavily furnished or electronic-rich areas, may experience elevated exposures through ingestion and dermal contact. Flame retardants have been detected in ship wastewater, suggesting potential for indoor dust as a vector for chemical contamination in marine environments. Strict performance-based fire test procedures are mandatory for all products onboard ships, but no regulations exist concerning the type of FR used or the concentrations thereof. These findings underscore the need for targeted regulation and further monitoring of chemical exposures in maritime environments, especially given the extended periods that crew members spend onboard.

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3.07.P-We231 Monitoring of Contaminants of Environmental Concerns along the coastlines of the Mediterranean Sea: the ONE-BLUE project

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The Mediterranean Sea is a semi-enclosed basin that is exposed to intense anthropogenic pressure. The presence of emerging contaminants in this area is a growing concern for the integrity of the ecosystem and human wellbeing. The continuous input of pollutants from coastal urbanisation, industrial activities, agriculture, maritime transport and tourism creates a dynamic and complex contamination scenario, in which many of these pollutants are not well characterised. In this context, the ONE-BLUE project aims to strengthen transnational knowledge and monitoring capacity by evaluating the occurrence and distribution of emerging contaminants across key sub-regions of the Mediterranean.

The study addresses the challenges of identifying priority contaminants, understanding their spatial patterns and supporting the development of harmonised monitoring strategies. To this end, surface and column water and sediment samples were collected in six representative Mediterranean areas: the Spanish coast, the Tyrrhenian Sea, the Ionian Sea, the Adriatic Sea and the Aegean Sea. Samples were extracted on board or in temporary laboratories near the sampling areas within 24 hours of collection to minimise degradation and ensure analytical reliability. The extracts were analysed using liquid chromatography coupled with mass spectrometry (LC-MS) to quantify a wide range of emerging pollutants, including pharmaceuticals, residues of personal care products, industrial chemicals, and other markers of human activity.

Overall, the ONE-BLUE investigation will provide a valuable evidence base for assessing environmental risk, guiding mitigation efforts, and informing policymakers on priority substances requiring targeted management.

Disclaimer/Disclosure:

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3.07.P-We232 Behaviour-based estimates of sunscreen-derived UV filter loads to a subtropical coral-reef beach in Okinawa, Japan

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Organic ultraviolet (UV) filters in recreational sunscreens are of concern because of their potential impacts on coastal marine ecosystems, yet quantitative links between beachgoer behaviour and UV filter inputs to seawater remain poorly constrained. Building on a monitoring campaign at a subtropical coral-reef beach in Okinawa, Japan, in summer 2024, in which we combined seawater sampling, passive samplers and a questionnaire survey, we extended this approach with a second, more detailed survey in summer 2025. In 2024, grab samples from three nearshore stations showed benzophenone-3 (BP-3, oxybenzone) concentrations up to 2.1 ng L⁻¹, with temporal changes that co-varied with bather density, tidal height and wind direction. Questionnaire responses from 169 beachgoers indicated that 75.7% used sunscreen and that six organic UV filters were present in 23 of the 28 identified products.

In 2025, we conducted structured face-to-face interviews with 302 beachgoers at the same site to obtain detailed information on sunscreen use, including frequency of application, treated body areas, product types and listed

ingredients. These data, together with time-resolved bather counts and beach geometry, were used to estimate the daily mass of sunscreen applied and the potential mass of each UV filter released to the bathing area by adapting the mass-balance framework of Labille et al. (2020), which links consumer survey data to UV filter loads and predicted environmental concentrations in beach waters.

We compare the estimated daily UV filter loads and predicted nearshore concentrations for the two survey days (2024 and 2025) and illustrate interannual and diurnal variability at this single coral-reef beach. The results provide an initial, behaviour-based quantification of sunscreen-derived UV filter inputs for Okinawa and a transferable framework to support future exposure assessments and the design of risk management options for coastal marine environments.

3.07.P-We233 Tracing the Unseen Journey of PFAS in Coastal Aerosols: Evidence from Atmospheric Deposition Studies at the Danish North Sea Coast

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Per- and polyfluoroalkyl substances (PFAS) are persistent and globally distributed contaminants whose environmental cycling remains unresolved. Atmospheric transport via sea spray aerosol (SSA), droplets ejected from breaking waves, may deliver PFAS from the ocean into terrestrial environments. This study investigates whether SSA drives PFAS pollution along the western North Sea coast of Denmark, where elevated PFAS levels are regularly observed in groundwater despite the absence of identifiable local sources. It is hypothesized that PFAS concentrations in atmospheric deposition decline inland, shaped by dry deposition of large SSA particles near the coast, consistent with the North Sea as the source.

A six-month field campaign along two transects extending 1 km inland employed automated wet/dry deposition samplers and bulk deposition collectors to capture continuous atmospheric PFAS inputs. Deposition chemistry was analyzed alongside dissolved organic matter signatures, meteorological observations, and nearshore aerosol particle-size distributions to characterize marine-derived contributions. Preliminary analysis of bulk deposition samples collected in November 2024 revealed an exponential decline in perfluoroalkyl carboxylic acid (PFCA) concentrations from the shoreline to 600 m inland, matching the decrease in sodium used as sea-salt tracer. PFCA concentrations reached 70 ng L⁻¹ at the coast, with perfluorooctanoic acid (PFOA) as the dominant contributor.

Although sampling is ongoing, these results already show that PFAS from the ocean can be transported inland via SSA, revealing a previously underappreciated pathway for coastal contamination. This has important implications for freshwater management, human exposure assessment, and future regulatory strategies.

3.07.P-We234 Mapping Organic and Inorganic Chemical Contamination Across the European Coastline

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Chemical compounds have enhanced our quality of life, however the pollution they generate has the potential to negatively impact both the environment and human health. The chemical exposome associated with human activities includes a wide array of substances, which can be grouped into two main categories, organic and inorganic chemicals which could then both be subdivided into legacy and contaminants of emerging concern (CEC). The Traversing European Coastlines (TREC) expedition is a pan-European survey that aims to extensively study coastal ecosystems and their responses to environmental changes and pollution pressures. A collaboration has been set up between TREC and CONTRAST, a Horizon Europe project aiming to understand how chemicals of emerging concern (CECs) may harm marine ecosystems and develop solutions to reduce their impact.

The objective of the current study is to map the chemical contamination (organic and inorganic) in European coastal surface sediments. Twenty-four sampling sites across the European coastline were selected (North-east Atlantic and Mediterranean seas). An extensive list of contaminants has been monitored and analysed, including i) both legacy organic contaminants and CEC (Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Polybrominated Diphenyl Ethers (PBDEs), Per- and Polyfluoroalkyls Substances (PFAS), Organophosphate Esters (OPE)), ii) trace metals and rare-earth elements (REEs), as well as iii) 64 unregulated CEC recently prioritized by Yulikayani et al., (2025).

We hypothesized that the contamination profiles would depend on the sampling site and nearby history of anthropogenic activities (estuary, national parks...). Indeed, some sites show a profile dominated by PAH

contamination due to their proximity to refineries and ports while others indicate a profile dominated by PFAS probably due to their proximity to industrial sites. There are also some hotspots of metal contamination found in sediments likely due to nearby mining or industrial activities. Besides, sediment properties (grain size, carbon organic) played an important role in the substance detection, highlighting the need for further adjustments in the sampling strategy to optimise the spatial scale assessment.

This study represents the first continent-wide initiative of its kind. It is then planned to link these contamination profiles to their impact on marine ecosystems biodiversity and functions.

3.07.P-We235 Fate and mobility of whale-watching vessel emissions in the marine environment and their effect on baleen whale behavior

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The whale watching (WW) industry has been significantly contributing to coastal economic growth and fostering public engagement in marine life conservation worldwide. However, the gradual expansion of this industry has intensified environmental stress in nearshore waters, used as potential feeding zones for baleen whales (BW). Although traditional WW vessels (diesel-powered) emit harmful atmospheric and underwater emissions that adversely affect the marine environment and cetacean behavior, their associated ecological and biological risks have been inadequately quantified in previous studies. Hence, this research investigates how vessel-derived emissions disperse and accumulate under oceanographic conditions and alter respiratory and acoustic behavior in targeted BW populations in northern Iceland using the case study method. A medium-sized traditional WW vessel sailing across the Skjálfandi Bay was selected as a case ship, and its technical specification and voyaging information were obtained from its operating company and port authorities to illustrate its voyaging interface by using Seamatrix (v. 2.5.0.0) software. The STEAM model was used to calculate different particulate and gaseous emissions, and their hydrodynamic dispersion in oceanic water was illustrated using ChemicalDrift (v. 1.0) software. Finally, behavioral observations of BW towards spatial emission footprints were evaluated based on surfacing-respiration data derived from secondary literature. The preliminary results depict that different vessel-derived emissions influence the water chemistry of the nearby shore, and their dispersion in oceanic water moderately overlaps with whale-sighting hotspots. Stratified water columns and wind direction have a significant influence on pollutant persistence in feeding grounds, which elevates respiratory loads and reduces surface time in BW species. The cumulative load-level exposure may vary between peak and off-peak tour seasons, which influences habitat use and foraging efficiency. The analysis indicated that vessel-derived emissions transport a considerable amount of chemicals and non-indigenous species in ocean water, resulting in numerous environmental impacts and behavioral disruption in marine mammals. Therefore, further research is warranted to understand the transport mechanism of these pollutants while determining threshold limits for biological exposure to improve sustainable WW practices.

3.07.P-We236 Life-Long Exposure to Lower Water Quality and Enhanced Contaminant Risk May Enhance Coral Tolerance to Zinc

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Tropical coastal mosaics of mangrove, seagrass, and coral reefs underpin biodiversity, carbon storage, shoreline protection, fisheries, and tourism. Yet climate change, coastal development, and declining water quality increasingly threaten these systems, especially in semi-enclosed bays where pollutant loads and ecological stressors can be amplified. Despite the ecological and socio-economic importance of these habitats, the effects of coastal contaminants on marine foundation species such as reef-building corals remain severely understudied. Here, we report unexpected yet ecotoxicologically informative findings from an experiment originally designed to test climate change tolerance in the branching coral species *Porites furcata*. Colonies were collected from two contrasting habitats in Curaçao (southern Caribbean): a semi-enclosed inland bay characterized by elevated nutrients and heightened ecotoxicological risk; and a nearby fringing reef with high water quality. During the acclimatization phase in an indoor aquarium facility, we observed paling and declines in photochemical efficiency (Fv/Fm) that were most pronounced in corals from the fringing reef (-53% compared to -17% in bay-origin corals). While these changes were initially attributed to acclimation to artificial lighting, subsequent tests revealed elevated zinc concentrations (~1.57 mg L⁻¹) in the seawater supplying the facility, consistent with known negative effects of Zn on photosynthetic processes in corals.

Corals were then transferred to artificial seawater containing only trace amounts of Zn. Fv/Fm values fully recovered within 2 weeks in both reef and bay-origin corals, confirming that Zn had likely caused the stress response. While we did not directly measure seawater Zn concentrations at the corals' collection sites, other

work has shown strongly elevated Zn concentrations in seagrass leaves in the bay. These results indicate that corals chronically exposed to heightened contaminant risk, including Zn, may develop enhanced tolerance to Zn. This suggests that Zn-tolerant coral populations may also occur in other semi-enclosed coastal environments with similar local stressors. Our findings highlight the need for systematic monitoring of Zn and other trace metals in tropical bays and for standardized toxicity tests across additional coral species to better understand Zn tolerance and its implications for reef ecosystem resilience.

3.07.P-We237 Assessing the Occurrence and Drivers of Organic UV Blockers and Titanium in South-western European Coastal Waters

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A large proportion of commercial sunscreens still relies on organic UV-filters, which have raised health concerns due to dermal absorption, detection in human milk, and ecological effects such as endocrine disruption. Their use has therefore been restricted in sensitive marine ecosystems. Titanium (Ti) and titanium dioxide nanoparticles (TiO₂ NPs) are increasingly promoted as “safe mineral UV-blockers”, yet their environmental risks remain poorly understood. Although Ti has been banned as a food additive in the EU, no regulatory limits currently exist for environmental concentrations of UV-filters or TiO₂ NPs, whose behavior is strongly influenced by natural factors such as salinity and organic matter. This study investigates the occurrence and co-distribution of organic UV-filters, Ti, and TiO₂ NPs along the southwestern European coast, aiming to identify key anthropogenic sources and environmental drivers shaping their presence.

Sampling was conducted in Summer and Autumn 2025 across three regions: Minho River, Ria de Aveiro, and Algarve, spanning nearly 1000 km and covering salinity gradients from freshwater to marine conditions. Ti and TiO₂ NPs were analyzed by ICP-MS and sp-ICP-MS, and organic UV-filters by LC-HRMS.

Preliminary summer results show relatively consistent coastal Ti levels (approx. 20 µg/L) and reveal strong salinity-driven aggregation of TiO₂ NPs, resulting in their low detection in marine waters. Our findings suggest that non-cosmetic Ti sources (particularly industrial activity and population density) may significantly contribute to environmental loads and pose potential risks to both ecosystems and consumers. Further ongoing assessment will support the development of monitoring strategies and regulatory frameworks for UV-filters.

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3.07.P-We238 Optimization of a Method to Assess the Exposure to Emerging Pesticides in the Atlantic Blue Crab Inhabiting a Threatened Sea Lagoon

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Coastal ecosystems are increasingly impacted by chemical pollution, yet monitoring programs rarely include crustaceans despite their ecological relevance and capacity to accumulate contaminants. The Atlantic blue crab (*Callinectes sapidus*), an invasive species abundant in the Mar Menor Lagoon (southeastern Spain), represents a promising bioindicator for assessing exposure to contaminants of emerging concern (CECs). This study aimed to optimize an analytical method to quantify 24 emerging pesticides in crab tissues using minimal sample amounts. Haemolymph, muscle, and hepatopancreas samples were extracted following a modified protocol and analyzed by HPLC–MS/MS. Method validation demonstrated optimal recovery, repeatability, and linearity for 21 compounds across all matrices, although strong matrix effects were identified for several pesticides,

requiring matrix-matched calibration. The method was successfully applied to crabs experimentally exposed to acetamiprid and imidacloprid, with both insecticides detected in all tissues, showing highest accumulation in the hepatopancreas. These results confirm that the optimized method is robust, sensitive, and well suited for monitoring emerging pesticides in blue crabs, offering a valuable tool for future environmental assessments in threatened coastal ecosystems.

3.07.P-We239 An integrated modelling and monitoring study on the sources, pathways, and environmental fate of pharmaceuticals in the Northern Adriatic Sea

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Chemical contamination by active pharmaceutical ingredients (APIs) has become a global environmental concern, as these compounds are only partially removed by conventional treatments and persist at low concentrations, affecting aquatic ecosystems and potentially human health. Their behaviour in coastal systems—especially semi-enclosed basins like the Northern Adriatic Sea—remains poorly understood despite high anthropogenic pressure and likely continuous inputs from rivers and wastewater.

This study quantified four key APIs—Trimethoprim (TRI), Clindamycin (CLI), Ofloxacin (OFL), and Venlafaxine (VEN)—in water and sediments by combining high-resolution modelling with seasonal monitoring. API emissions were estimated using regional consumption data, accounting for human and veterinary use and removal efficiencies. Water quality simulations were performed with the ChemicalDrift model, forced with hydrodynamic fields from SHYFEM and CMEMS. Four seasonal sampling campaigns provided data for quantitative UHPLC–MS/MS analysis.

TRI showed the highest annual load, followed by CLI, VEN, and OFL. The Po River contributed over 60% of total inputs, with secondary contributions from the Adige, Gorzone, Bacchiglione and Reno rivers, reflecting differences in local population and livestock distribution.

Model results revealed significant temporal variability in water concentrations, with localized peaks near the shore and lower backgrounds in the open sea. In some detail, CLI concentrations in water reached up to 2000 pg·L⁻¹, while TRI and VEN concentrations were generally lower (ca. 100 pg·L⁻¹), and OFL stayed mostly below 2 pg·L⁻¹. Sediment accumulation was limited to TRI and CLI, while VEN and OFL were modelled to remain largely dissolved. Spatial patterns showed consistently higher concentrations near river deltas and wastewater discharge zones, indicating dominant land-based inputs, while offshore waters and sediments exhibited low and levels of contamination.

Overall, this work highlighted the persistence, variability, and spatial heterogeneity of API contamination in the Northern Adriatic Sea, showing how the integration of modelling and monitoring can support the development of effective management strategies for the control of these chemicals' emissions.

Disclaimer/Disclosure:

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3.07.P-We240 Use of Sea Urchin Liquid-phase Bioassays for the Ecotoxicological Analysis of Construction Products in Coastal Environments

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The increasing incorporation of industrial by-products and waste-derived components into construction materials raises pressing concerns regarding their potential ecotoxicological impact on vulnerable coastal environments. These areas, situated at the intersection of human activities and open ocean dynamics, are particularly exposed to chemical leaching processes that may compromise water quality and ecosystem integrity. Within this context, standardized bioassays using marine model organisms provide a robust framework to translate chemical complexity into ecologically relevant endpoints.

In this study, we evaluated the ecotoxicity of leachates from a standardized cement (CEM V/B) incorporating blast furnace slag and metakaolin, using equilibrium leaching tests (EN 12457-1/2) at liquid-to-solid ratios of 2

and 10 L/kg. Two complementary bioassays were applied: the luminescence inhibition test with *Vibrio fischeri* and the embryonic-larval development assay with the sea urchin *Paracentrotus lividus*. Results revealed a marked influence of leachate concentration and L/S ratio on toxicity outcomes. While the bacterial assay indicated significant luminescence reduction, exceeding the toxicity threshold defined by CEN/TS 17459 at LS2 (LID = 18.83), the sea urchin assay provided a more sensitive and ecologically integrative perspective. Larvae exposed to LS10 leachates consistently reached stage 2 (four-leg development), whereas LS2 leachates at high concentrations (?75%) severely inhibited growth initiation, with significant deviations from control populations. These findings underscore the limitations of single-test approaches and highlight the necessity of multitrophic bioassay batteries for comprehensive risk assessment. The inclusion of sea urchin embryogenesis assays, a well-established marine model system, expands the interpretative scope beyond microbial endpoints, capturing developmental disruptions that are critical for population sustainability. By integrating chemical analysis with biological responses across trophic levels, this work demonstrates the relevance of harmonized ecotoxicological tools for evaluating innovative construction products in coastal zones. Ultimately, the study contributes to the advancement of standardized test systems that safeguard marine ecosystems against diffuse pollution sources, aligning with European guidelines (CEN/TR 17105) and reinforcing the role of ecotoxicology in bridging human activities with ocean health.

3.07.P-We241 Oysters as pollution sentinels in a Basque estuary (Spain): seasonal and metabolic variability

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The use of sentinel species sensitive to chemical pollution is key to capture biological impacts in environmental monitoring programs. In particular, filter feeders are known to bioaccumulate contaminants and precisely reflect broad exposure periods in coastal areas. In this study, oysters from a moderately impacted estuary in the Basque Country (N Spain) were collected seasonally between 2023 and 2024. Oysters are considered potential substitutes for mussels as sentinel organisms in monitoring programs in this area. Therefore, this study aims to assess bioaccumulation and the occurrence of chemical contaminants in this species to correlate it with its health status. 30 oysters were collected seasonally at 4 points along the estuary, from the inner area already influenced by anthropogenic sources such as wastewater, until the mouth of the estuary on a saline environment, and biometric data was retrieved. Then, they were pooled together, freeze-dried and homogenized for the analysis of legacy and emerging semi-volatile pollutants including 16 polycyclic aromatic hydrocarbons, 8 polychlorinated biphenyls, 6 polybromated biphenyls, 34 pesticides, 6 UV filters, 7 phthalates and 8 fragrances. Samples were extracted using focused ultrasonic solid-liquid extraction, purified using 15g florisil columns and injected onto an Agilent 7890A gas chromatograph coupled to an Agilent 5975C quadrupole mass spectrometer. Preliminary results indicate positive detection of many legacy (PAHs) and emerging chemicals (UV filters, among others), suggesting relevant ecological damage as previously observed with the disappearance of other local filter feeders like mussels. The correlation between seasonal and spatial patterns will be explored willing to elucidate the insights on exposure and the biological cycles.

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3.07.P-We242 Conifer Resin Acids as Indicators for Ship Coatings' Environmental Impact

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Most ships use selfpolishing antifouling coatings (SPCs), which erode evenly over time to prevent biofouling and maintain a smooth coating surface to lower fuel consumption. However, ship coatings remain an underestimated source of microplastics, nanoplastics, and associated additives (MNP&A) in the marine environment. Especially near-shore areas with strong nautical impact may experience a significant input of SPC-particles to MNP&A. To classify SPC contribution to the marine MNP load and estimate environmental impacts, a qualitative and ideally quantitative measurement variable is prerequisite.

To identify SPC-particles in the environment, a set of chemical markers commonly used across SPC products is needed. SPCs are typically based on hydrolyzable copolymers incorporating high amounts of conifer resin-related compounds (RRCs) which are either covalently or non-covalently bound. They mainly consist of natural and modified diterpene resin acids with known ecotoxicological potential and are released as SPCs disintegrate. This projects' aim is to elucidate SPC-based MNP&A input into the marine environment by i) evaluating SPCs high RRC content to find suitable chemical markers for released particles and ii) characterize leaching-based input of RRCs as potentially harmful additives.

A set of 4 representative, uncured SPC products was separated into solubility fractions to isolate potential leachate and precipitated polymers, analyzed via GC-MS and Py-GC/MS, respectively. Additional cured SPC-particles were generated and analyzed via Py-GC/MS. Finally, environmental samples (water, sediment, biota) analyzed for MNP via Py-GC/MS were screened to detect selected RRCs. Considering further leaching of RRCs as SPC-particles age, ongoing laboratory experiments are used to induce hydrolysis.

Whith one exeption, RRC composition was highly consistent across SPCs. Several RRCs were selected as potential SPC markers and partially tentatively identified via NIST library. Unknown RRCs were grouped based on key fragment ions, whith structure elucidation still ongoing. High levels of RRCs were found in both leachate and polymer binder fraction isolated from uncured SPCs. First experiments show that hydrolysis does not release all RRCs from the isolated polymer binder. A set of determined RRCs was detected in marine MNP samples (water, sediment, biota). Our preliminary results underscore RRCs' potential in unravelling the impact of ship coatings to marine MNP&A pollution.

3.08 Emerging Chemical and Microbial Threats to Drinking Water Quality: Identification, Prioritization and Risk assessment

3.08.T-01 Persistent DOM Fractions as Dominant Precursors of DBP Formation: Integrated Target and Non-Target HRMS Insights from a Full-Scale DWTP

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Chlorination remains the most widely used disinfection method in drinking water treatment due to its simplicity and cost-efficiency. However, reactions between chlorine-based disinfectants and dissolved organic matter (DOM) generate disinfection byproducts (DBPs), many of which remain poorly characterized, unregulated and potentially more toxic than those currently controlled (THMs, HAAs). In this study, we investigate DBP precursor behaviour and formation in a full-scale DWTP by integrating a multi-analytical approach based on (i) target analysis of 17 regulated and emerging DBPs, (ii) bulk measurements of adsorbable organic halides (AOX) and (iii) non-target DOM fingerprinting by high-resolution mass spectrometry (HRMS).

To assess the performance of the treatment system and the evolution of water quality throughout the distribution network, seven sampling points were selected, four treatment stages and three distribution systems. To capture seasonal variability and reduce sampling bias, six sampling campaigns were performed during three seasons (spring, autumn and winter) with two sampling events per season.

Twelve of the 17 target DBPs (four THMs, three HANs, five HAAs) were detected in finished water, with concentration ranges of 13–42 µg/L, 1–4 µg/L and 6–13 µg/L, respectively. HRMS showed that aromatic and condensed-aromatic DOM was efficiently removed, whereas aliphatic and unsaturated-like fractions persisted along the treatment train and accumulated newly formed halogenated species. AOX profiles, dominated by chlorinated organics, revealed a large pool of unknown halogenated compounds, consistent with low-oxygen unsaturated formulas in the HRMS fingerprints.

A Spearman correlation analysis highlighted 97 formulas strongly associated with DBP formation, nearly 60% of which persisted through all treatment stages. These precursors were enriched in aliphatic and low- or high-oxygen unsaturated-like van Krevelen regions; the fractions least removed by conventional treatment. Overall, our findings demonstrate that DBP formation is controlled by a narrow, structurally resilient subset of DOM. This work provides a transferable, molecular-level framework to move from bulk DOM removal towards targeted treatment strategies that selectively remove persistent DBP precursors and thereby enhance drinking water safety.

Disclaimer/Disclosure:

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3.08.T-02 A plasmonic nano-tastebud sensor for the detection and discrimination of organic micropollutants in drinking water

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Monitoring organic micropollutants (OMPs) in drinking water is still limited by slow, laboratory-based LC-MS/MS methods that cannot provide real-time information, especially in decentralised or rapidly changing systems. To address this gap, we developed a cross-reactive plasmonic “nano-tastebud” sensor that detects changes in water chemistry through multidimensional optical fingerprints rather than targeted analyte recognition.

The platform integrates microfluidics with 24 functionalised plasmonic metasurfaces whose localised surface plasmon resonance (LSPR) shifts respond to supramolecular interactions with waterborne compounds. We tested 11 representative OMPs, including estrogens, painkillers, antibiotics, anti-diabetics, stimulants and pesticides, spiked into tap water from 10 ng L⁻¹ to 1 mg L⁻¹. Optical responses were processed using PQN normalisation and evaluated through multivariate and machine-learning models (PCA, LDA and random forest). Independent-run validation was used to assess real-world robustness, and DOC was included as an auxiliary predictor.

The sensor consistently detected chemical changes at concentrations as low as 1 µg L⁻¹ and produced reproducible fingerprints that allowed discrimination of pollutant classes. Estrogens and painkillers generated distinctive, high-intensity responses, making them strong indicators of wastewater-related contamination. Other pollutant groups produced subtler signals but remained distinguishable from baseline water. Importantly, the platform generalised well across runs, demonstrating resilience to day-to-day drift and matrix variability, critical for field deployment.

This study provides the first evidence that a nano-plasmonic cross-reactive sensor array can rapidly detect and differentiate mixtures of OMPs in drinking water without relying on selective receptors. The approach offers a fast, field-deployable tool for early-warning detection and operational monitoring and has strong potential to complement or reduce reliance on conventional LC-MS/MS in decentralised water systems.

3.08.T-03 Exploring biodegradation potential of nitramines in drinking water, biproducts of amine-based carbon capture.

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Amine-based carbon capture plants (CCPs) can release amines that transform into carcinogenic nitrosamines (NSAs) and nitramines (NAs), potentially contaminating drinking water sources. In Norway, the strict safety threshold of 4 ng/L for these compounds poses a significant analytical challenge, making environmental modeling essential for risk assessment. Accurate models rely on precise degradation kinetics with mechanisms differing between NSAs and NAs. While photodegradation drives NSA removal, biodegradation is the dominant pathway for NAs.

This study assessed biodegradation of three representative nitramines—MEA (N-(2-Hydroxyethyl)nitramide), AMP-NO₂ (N-(1-Hydroxy-2-methylpropan-2-yl)nitramide), and NIPZ (1-Nitropiperazine)—using a modified OECD 309 test with water and sediment from Lake Elvåga, a drinking water source near a planned CCP. Experiments compared the natural attenuation in water, water-sediment inocula against adapted inocula under nitrogen-limited conditions. Primary degradation was monitored by LC-MS/MS, while microbial community changes were analyzed via flow cytometry and 16S rRNA sequencing.

Biodegradation rates proved highly compound depending: MEA degraded rapidly (DT₅₀: 7–34 days), AMP-NO₂ showed moderately (DT₅₀: 12–40 days), while NIPZ showed negligible degradation (>60 days). Sediment

addition significantly enhanced degradation compared to water alone, whereas prior adaptation offered limited increase in degradation. Abiotic processes contributed to MEA degradation in sediment. Microbial analysis revealed a decline in diversity under nitramine and phosphate exposure, yet identified no universal nitramine-degrading bacteria.

These results underscore that amine selection in CCP design is critical for mitigating drinking water risks. Furthermore, the findings suggest that microbial diversity plays a key role in regulating the persistence and degradation of nitramines in polluted recipients. Collectively, this study provides essential kinetic data for regulatory modeling.

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3.08.T-04 Semi-Automated Workflow for the Identification and Screening of Active Degradation Products of Tyrosine Kinase Inhibitors After Treatment with Aerobic Activated Sludge

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Routine monitoring of drinking water sources for contaminants is commonly limited to the determination of known priority substances through chemical analysis. During the treatment of wastewater, chemicals can be transformed into other potentially hazardous and/or more mobile degradation products that remain undetected. Efforts to keep track of potential hazardous compounds released into the environment and to monitor the processes of drinking water purification depend on the ability to timely identify these compounds. This is especially important for novel pharmaceuticals with unknown impact human health. One relatively novel, expanding and structurally diverse class of (increasingly prescribed, anti-cancer) pharmaceuticals are tyrosine kinase inhibitors (TKIs). Determination of their impact depends on insight into their fate (e.g as degradation products). The advent of high-throughput effect-directed analysis (HT-EDA) provided a strategy to identify novel toxicants in mixtures by combining effect-based methods with analytical identification methods. However, HT-EDA lacks compatible bioassays for TKI screening and remains a laborious method with low identification success rate. To achieve streamlined formation, detection and improved identification of (active) TKI degradation products, an integrated workflow was developed through application of lab-scale degradations with a semi-automated identification workflow and enzymatic kinase inhibition assays. The applicability of this approach will be demonstrated by analysis of a selection of TKIs prescribed in The Netherlands. The application of lab-scale degradation together with specialized non-target screening workflows and high-resolution effect-based testing will open the way to preemptive identification of degradation products as emerging contaminants and will emphasize the importance of residues when considering environmental contaminant impacts.

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3.08.T-05 Assessment of Health Risks Associated with Soluble Polymer Application in Drinking Water Production

Bianca van de Ven and Dominique Narain-Ford, RIVM, Netherlands

Synthetic polymers play an essential role in modern water treatment processes, where they are used to improve the quality and safety of drinking water. While much attention has been given to the environmental impact of insoluble polymers and microplastics, less is known about the potential human health risks associated with water-soluble polymers used in drinking water production.

This study examines the application and health risk assessment of water-soluble polymers in drinking water production. To establish a comprehensive overview, all active and recently expired certificates from the KIWA database were systematically reviewed, covering 100 dossiers. The investigation focused on polymers functioning as antiscalants, antifoaming agents, and conditioning agents. Relevant legal frameworks and guidelines were assessed to position current practices within the regulatory context.

Each polymer identified was evaluated for persistence, mobility, and toxicity (PMT) properties, with an emphasis on parameters pertinent to human health risk assessment. Data collection included both certificate information and supplementary literature review. Where available, EFSA opinions regarding food additives were consulted to support the risk estimation.

The results identified 18 distinct polymers distributed across 28 certificates, most commonly used as antiscalants. These polymers generally exhibit polydisperse molecular weight distributions and are tailored for specific roles in water treatment. For five polymers, PM properties could be identified using QSAR models; however, for the majority, the PMT/PBT scoring approach based on QSARs proved unsuitable due to identity challenges and data limitations. Additionally, the study considered potential human exposure from residual polymer content and migration from materials, particularly during the initial months following installation. These findings provide a foundation for improved human health risk assessment of water-soluble polymers in drinking water production and highlight the need for more targeted research and regulatory attention in this area.

3.08.P Emerging Chemical and Microbial Threats to Drinking Water Quality: Identification, Prioritization and Risk assessment

3.08.P-Tu209 Identification and Prioritisation of Emerging Pollutant Sources Using AI and GIS to Protect Drinking Water Wells

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The water sector in the Netherlands faces increasing challenges due to emerging pollutants, particularly substances of very high concern (SVHCs), in waterbodies, including drinking water wells. As of January 2025, around 3,200 SVHCs and 400 potential new SVHCs have been identified. Governmental organizations aim to create a safe environment by reducing SVHCs from industrial activities. However, manual identification of sources and monitoring is time-consuming and resource-intensive, hindering effective management. This study leverages GIS datasets, AI, and FME scripts to enhance the detection and mapping of potential SVHC sources. Using AI and data-driven techniques, it assesses industrial activities and their emissions to help prioritize specific sources and develop mitigation strategies. To pinpoint industrial sources of SVHCs, FME scripts and filtering techniques were applied to the Dutch Chamber of Commerce (CoC) dataset. This dataset includes over 700,000 businesses within an area of interest of approximately 410 km². Multiple filters, derived from National Institute for Public Health and the Environment studies and expert input from in-house projects, helped identify businesses that could be potential sources of SVHCs. Non industrial sources such as (former)landfills were identified using open data and large language models for locations where firefighting foams were used. GIS is used for visualization to identify and prioritize SVHC sources near drinking water wells. A tailor made risk-matrix was used to score each source in order to prioritize them for policy making in order to protect nearby drinking water wells. The integration of multiple data sources and filtering techniques in an automated GIS workflow allowed a targeted identification of contamination hotspots. An online GIS-based dashboard provides policymakers easy access to centralized, relevant data, enhancing decision-making and response times. The study demonstrates the effectiveness of using large GIS datasets for identifying potential SVHC source locations. The centralized GIS dashboard improves efficiency and aids policymakers in making informed decisions. Future work includes adding historical SVHC measurements and developing real-time monitoring tool. The findings emphasize the importance of data integration and spatial analysis in environmental risk assessment, providing a robust framework for future investigations and helping protect drinking water wells effectively.

3.08.P-Tu210 Characterization of thyroid hormone-replacing activity in surface water using effect-directed analysis.

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Surface waters are exposed to mixtures of chemicals, many chemicals of which have been found to inhibit thyroid hormones (THs) such as thyroxine from binding to transport protein transthyretin, also called TH-displacing activity. The potential impact of TH-displacing activity on human health and the environment is of growing concern. Effect-based methods, such as in vitro bioassays, have revealed TH-displacing activity in various water matrices. Indeed, our studies have shown that TH-displacing activity is present in wastewater, surface water and also in drinking water. The environmental effect-based trigger value (EBT) for TH-displacing activity is often exceeded in surface water, indicating potential environmental risk. The chemical drivers behind this activity, however, remain largely unidentified.

This study aimed to further characterize TH-displacing activity in surface water that were known to be

contaminated with PFAS, with the expectation that we might be able to explain a large portion or even fully explain TH-displacing activity by target PFAS. Indeed, the contribution of target PFAS to TH-displacing activity was higher than found in our previous study [3]. Effect-directed analysis (EDA) was applied with nontarget screening (NTS) to identify bioactive compounds that contributed to TH-displacing activity in water to further close the gap. EDA led to the tentative identification of 12 compounds, including plant protection products and carboxylic acids, with four confirmed as contributors to TH-displacing activity. Further identification of bioactive compounds is ongoing using non-target approaches such as MS2 matching of unidentified features to those that were identified and Kendrick Mass Defect analysis. Moreover, a risk assessment will be performed on the known bioactive compounds in surface water to assess if there indeed exists an environmental risk upon exceedance of the EBT.

Ultimately, this research provides guidance to water quality managers and drinking water companies on how the FITC-T4 bioassay can be used to complement water quality monitoring for assessing environmental risk. Moreover, whether an operational response is necessary upon exceedance of the EBT to ultimately protect human health and the environment.

3.08.P-Tu212 Odour Deviations in Drinking Water Induced by Pressure Changes in the Distribution Network

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Drinking water providers ensure safe and pleasant drinking water through advanced treatment processes and stringent quality control. However, they sometimes receive complaints about taste and odour (T&O) in the drinking water. Since T&O are key characteristics on which people assess drinking water quality and safety, badly perceived T&O events decrease consumers' confidence in drinking water quality and cause people to choose bottled water over tap water. Production and/or release of T&O compounds during drinking water distribution should be avoided as the water is directly delivered to consumers without further treatment. Odours can be caused by leaching of compounds from pipe materials, microbial metabolism, disinfection reactions, or diffusion of odour compounds or precursors from external sources. Increasing knowledge of T&O origins is needed to better solve future problems.

Therefore, this research focuses on odour deviations related to changes in pressure during maintenance work at the high-pressure (HP) drinking water distribution system. A trace analytical method targeting 45 T&O compounds is optimized by including a derivatisation step to improve halophenol detection/quantification. Next, in a field section of a real HP pipe (± 3 km), three scenarios were systematically investigated. Lowering the overpressure in the HP pipe from 10 to 5 bar resulted in an observed bitumen/asphalt odour, which further increased in intensity after a subsequent sudden pressure drop to 2 bar. Based on the odour threshold and profile, two halophenols were detected that may have contributed to this odour observation. When the section was partially emptied, 6 halophenols and 5 other T&O compounds were identified. Our results indicate that pressure changes induce the leaching of T&O compounds from sealing materials connecting the 6m-pipe sections. This is further supported by the detection of similar T&O compounds in a water extract of the sealing materials and in an odorous household sample collected after maintenance work in the HP-network.

Overall, this study is highly relevant for identifying and quantifying key T&O compounds and their sources in (HP) drinking water distribution networks. Next to the acquired scientific knowledge, our findings have direct operational relevance, as effective pressure management during maintenance works will be a key factor for drinking water companies in preserving water quality and minimise the risk of odour-related issues in DWDS.

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3.08.P-Tu213 Toxicity Prediction from MS2 Fingerprints to Support Feature Prioritization in Non-Target Screening

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Non-target screening (NTS) enables the detection of large numbers of unknown features in water samples, yet interpreting their potential relevance remains challenging when structural information is limited. This study explored whether fingerprints derived from tandem mass spectra (MS2), combined with machine-learning models, can support the prioritization of features detected in NTS workflows.

Groundwater samples were collected from two locations used for drinking-water production. Samples were analysed by hydrophilic interaction chromatography (HILIC) coupled to high-resolution mass spectrometry (HRMS). Features were extracted using patRoön followed by formula and candidate generation using SIRIUS and database searches. MS2 fingerprints were generated using SIRIUS+CSI:FingerID and subsequently used as inputs for two prediction models: MLin vitroTox and deepFPlearn.

A total of 590 features were detected at Location A and 395 at Location B, with 272 features shared across both locations. After quality filtering, MS2 data were available for 280 features, and fingerprints were obtained for 163 of them. MLin vitroTox classified 158 fingerprinted features as active on at least one endpoint, covering 51 mechanistic targets. DeepFPlearn identified 11 features with a probability above 0.5 for one or more of its evaluated endpoints. An example of these preliminary outputs is shown together with relative feature abundance, to explore whether the model predictions relate to the presence of these features in the samples. These findings represent an initial step toward integrating MS2-based predictions into NTS prioritization. Future work will incorporate concentration estimates and bioassay validation to refine and evaluate this workflow as a method for supporting risk-oriented interpretation of unknown features detected by NTS.

3.08.P-Tu214 Sample preparation determines chemical characterization and in vitro responses in water sources for drinking water supply

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Chemical micro-contaminants occur in surface waters as complex mixtures, yet conventional target analysis often fails to explain the biological effects observed in water used for drinking water production. Effect-directed analysis integrates advanced chemical and bioanalytical tools to better characterize these mixtures, but sample preparation remains a critical and frequently overlooked step influencing both analytical sensitivity and bioassay performance.

We evaluated three solid-phase extraction procedures covering broad chemical domains to determine their suitability for combined chemical and toxicological assessment of contaminants of emerging concern in water sources for drinking water supply. A sequential mixed-sorbent method was compared with single-sorbent methods using Oasis Hydrophilic-Lipophilic Balance and Mixed-Mode Cation Exchange cartridges. All methods were optimized and applied to grab samples from multiple abstraction points collected during two campaigns.

Chemical analysis by liquid chromatography coupled with high-resolution tandem mass spectrometry revealed marked differences in the number and type of compounds extracted among methods, with the mixed-sorbent procedure yielding the widest chemical coverage. Concentrations varied spatially and seasonally across sampling sites. In vitro bioassays assessing endocrine, metabolic, genotoxic and oxidative stress responses confirmed that all extracts elicited biological activity, but the magnitude and pattern of responses differed among extraction methods. Variability in metabolic and anti-androgenic activities was particularly pronounced, highlighting the impact of extraction selectivity on the observed toxicity profiles.

These results demonstrate that sample preparation strongly shapes both chemical and biological outcomes in quality assessment of drinking water sources. Ongoing data treatment will investigate the extent to which each extraction method explains observed biological activity and supports mixture toxicity interpretation. Findings from this work support the need for careful method selection to ensure robust and interpretable water quality assessments.

Disclaimer/Disclosure:

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3.08.P-Tu215 In silico toxicity assessment of data-poor substances migrating from polymer-based drinking water distribution materials

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Polymer-based materials, such as plastic pipes and connection parts, are widely used in drinking water distribution systems. Although safety-tested, studies show that trace amounts of chemicals can migrate from these materials into drinking water. For many of these substances, consumer exposure and potential human health or environmental risks remain unclear.

This research aimed to identify data-poor substances migrating from distribution materials and assess their mutagenic potential using in silico toxicity models. Ames bacterial mutagenicity was selected as the primary endpoint for its robust experimental data in the models used, improving prediction reliability.

A systematic literature review identified 276 distinct organic substances migrating from distribution materials, including additives, monomers, polymerization aids, and degradation products. Substances were prioritized based on legislative relevance – relating to the revised EU Drinking Water Directive – and structural alerts for mutagenicity, yielding 89 prioritized substances.

Bacterial mutagenicity predictions were generated using read-across in the OECD (Q)SAR Toolbox (v4.8) and three QSAR models from the bacterial mutagenicity model in MultiCase CASE Ultra software (GT1_BMUT, PHARM_BMUT, GT_EXPERT). Substances without experimental data (41 out of 89) received mutagenicity predictions. Among these, 26 received reliable unanimous negative predictions and are therefore unlikely to be mutagenic. None received reliable unanimous positive predictions; all positive outcomes were contradicted by at least one negative or inconclusive prediction, resulting in an overall inconclusive result. Fifteen substances had inconclusive results and were further investigated for external experimental mutagenicity data. They were categorized into high, medium, and low testing priority categories based on prediction outcomes severity to guide future testing efforts.

The findings of the current study suggest that current migration studies may overlook potentially hazardous substances due to their focus on targeted analytical methods and limited material types. The use of broader detection approaches, including non-target screening, and expanded sampling to include more material types and distribution-system-wide assessments are recommended to better capture real-world exposure.

This study was supported by the KWR Waterwijs program of Dutch and Flemish drinking water utilities.

3.08.P-Tu216 Migration of organic compounds from rubber materials in contact with water and their safety evaluation as water contact materials

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Water for human consumption comes into contact with different construction materials used in the collection, treatment, storage, and supply systems, and in the building networks up to the consumer's tap.

All the materials used in water supply structures have the potential to change water quality by leaching into the water, due to electrochemical mechanisms, biological phenomena, oxidation, corrosion, among others.

The nature of the leached compounds and the potential chemical interactions depend on the type of material used, namely, cementitious (cement, aggregates, water, additives, adjuvants, and fibers), organic (polymer matrix composites and elastomers, namely high-density polyethylene and epoxy resins) and metallic (especially stainless steel, copper, ductile iron, and metal alloys). These compounds can be harmful to human health.

The connection between the different pipework sections can be made through metal compression fittings, with the sealing being achieved by rubber sealing rings, through fittings of the same material connected by polyfusion welding.

In this work, six series of specific migrations studies from rubber materials intended for water contact have been studied. The amount of material used for each test depends on the surface-to-volume ratio (S/V) of each material and, the tests take place with a ratio S/V of 3 and 6. The experiments were carried out with ultrapure water under suitable conditions of time and temperature [1,2]. The liquid-liquid extraction of 500 mL of migration water in acid and basic conditions was used for the extraction of all potential organic compounds with 200 mL of dichloromethane. The organic extract was concentrated to 0.5 mL in a Turbovap system and analyzed by gas chromatography coupled to mass spectrometry [3]. A semi-quantitative method with ten deuterated standards was used for the quantification of all the compounds identified in migration waters. The electric conductivity, redox potential, color, pH, and the turbidity of migration waters were also performed.

More than 50 organic compounds were quantified in migration waters with a total concentration of up to 2608 µg/L. Most of the organic compounds showed concentrations lower than 10 µg/L, but some of them showed concentrations of up to 200 µg/L. The number of organic compounds and their concentrations decrease with the number of migration periods.

3.08.P-Tu217 Impact of Emerging Pollutants and Associated Health risks for Antibiotic-Resistant Bacteria and Resistance Genes in Urban Rivers

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Antibiotic resistance in aquatic systems is rapidly increasing due to the interaction of ARGs (Antibiotic-resistant genes) with numerous environmental stressors. The continuous discharge of sewage and other contaminants creates conditions that allow antibiotic-resistant bacteria (ARB) and their genes to thrive and spread. Strong correlations between faecal indicator bacteria and ARB indicate untreated waste inputs as major contributors of resistant bacteria in the environment. Surveillance from rivers like the Ganga and the Yamuna in India has shown the presence of potential pathogenic ARB such as *Pseudomonas*, *Acinetobacter*, *Aeromonas*, *Escherichia*, *Enterobacter*, and *Klebsiella*. However, the increasing burden of ARB in aquatic systems is compounded by heavy metal contamination, continuous sewage influx, and environmental stressors that intensify horizontal gene transfer and enrich high-risk clones. Drinking-water studies globally report WHO-priority ARB such as carbapenem-resistant *Klebsiella pneumoniae*, ESBL-producing *E. coli*, multidrug-resistant *Acinetobacter* spp., *Staphylococcus aureus*, *Salmonella*, *Shigella*, and *Streptococcus pneumoniae*, all associated with significant disease burdens. Yet, despite their widespread detection, risk assessment remains limited due to the absence of suitable antibiotic-resistant dose–response models (DRMs). Traditional DRMs treat pathogens as antibiotic-susceptible and fail to capture ARB dynamics, co-selection pressures, or realistic human exposure pathways. Recent advances propose a stochastic simple death (SD) process to model ARB and antibiotic-susceptible bacteria jointly, enabling incorporation of antibiotic concentration effects, horizontal gene transfer, mixed bacterial populations, and illness treatability. A case study using gentamicin-resistant and susceptible *E. coli* demonstrated how such models can more accurately predict health outcomes across antibiotic exposure gradients. A comprehensive, next-generation DRM that integrates environmental stressors, pollutant co-selection, ARG abundance, and human exposure scenarios—such as recreational, drinking-water, or incidental ingestion—would yield far more realistic infection risk estimates. Building upon recent model innovations, incorporating ARB behaviour directly into QMRA frameworks is essential for evaluating and mitigating AMR risks in polluted urban river systems.

3.09.A Environmental Forensics: Tracking Sources and Evaluating the Effects of Chemicals in Urban and Other Environments

3.09.A.T-01 The INQUIRE project: Human exposure to indoor house dust: characterization of unknown chemical compounds and their potential sources

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Homes are small ecosystems linked to the outside world while being vastly different due to individual behaviours. Since people spend a majority of their time indoors, increasing their likelihood of exposure to hazardous chemicals. Making the indoor environment of high importance to test.

The aim of this study was to identify potentially harmful chemicals and potentially sources of them in eight European countries. The matrix used for identifying chemicals is dust, due to its complex nature and as a source of exposure.

Nearly 300 dust samples were collected, extracted and analysed with liquid chromatography high-resolution trapped ion mobility spectrometry time-of-flight mass spectrometry (LC-TIMS-TOF) for both negative and positive mode. Resulting in the detection of more than twenty thousand chemicals varying from insect repellents to plasticizers.

Country-specific trends were found that highlight the importance of improving the indoor environment and how that is reflected in legislations, or the lack thereof. Additionally, sources of chemicals could potentially be identified by questionnaires about personal habits.

The link between chemicals in consumer products sampled from the houses as potential sources is studied aiming to improve the indoor air quality and finally through legislation.

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3.09.A.T-02 From Indoors to Surface Water: Tracking Contaminants Released From Textiles During Laundry via Target and Non-Target Screening

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A wide range of contaminants can be found in wastewater and surface water, of which many can contribute to mutagenic activity, but for which the sources are not well-understood. This is particularly true for indoor sources impacting on water quality. One possible pathway how indoor chemicals may reach water bodies is the emission via laundry. Accumulation of indoor contaminants on textiles and re-release via laundering was reported for phthalate esters, brominated flame retardants (BFRs) and organophosphate esters, but information for other chemical classes is scarce.

To characterise the exposure and sources of indoor contaminants in surface waters, we conducted a controlled indoor textile exposure study, from which textile extracts and laundry samples were generated. Indoor locations included (i) pubs where smoking is allowed, (ii) restaurant kitchens where most dishes consist of meat products, (iii) hair salons and (iv) flats where the residents are non-smokers and vegetarians/vegans. The samples were characterised using LC-HRMS/MS and evaluated using target and non-target screening approaches. Additionally, wastewater treatment plant (WWTP) influent and effluent samples were collected to trace the contaminants released from textiles further downstream.

Source-specific features that are present in the textile samples, representing the first step in the textile & laundry pathway were investigated. Unique features were then investigated in laundry and wastewater samples. The patterns from the textile samples can also be observed for laundry samples, albeit not for all features. From the 2335 unique features in smoking pubs filtered from textile samples 1186 were present in laundry and 329 were detected in wastewater influent. The unique features underwent hierarchical clustering to link samples that show similar occurrences, proving to be especially effective for smoking pub samples, where laundry and textile samples clustered together. Unique features that could be detected in textile, laundry, wastewater influent and effluent will be prioritised for identification using reference standards. The results will allow us not only to characterise indoor contaminants humans are exposed to from indoor environments but also to understand the extent of indoor environments as a source of contaminants in surface waters for better mitigation efforts to reduce surface water contamination.

Disclaimer/Disclosure:

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3.09.A.T-03 Associating point source PAH sources with vapor-phase Isotopic Carbon Ratios collected with Passive Sampling Devices

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Identifying a source of an environmental pollutant such as polycyclic aromatic hydrocarbons (PAHs) can be done using chemical ratios and pinpointing characteristic PAHs. However, these techniques can be influenced by chemical degradation and weathering over time. Stable isotopic ratios are less subject to these effects. We are creating an isotopic source library for carbon isotope data from passive sampling extracts with single-source contributions from common sources of PAHs such as natural gas, vehicle exhaust, wildfires, superfund sites, etc. We have shown that we can accurately measure a carbon isotopic ratio by PAH from low-density polyethylene (LDPE) passive sampling extracts. Now we continue to put together an isotopic library to aid in differentiating PAH sources in environmental mixtures. Passive sampler data as well as available standard reference materials were analyzed for 64 PAHs via GC/MS/MS. Initial sample analysis was performed on an

Agilent 7890 GC with a 7000 QQQ MS. Detected PAHs were used to create a time reference standard to provide a comparison in retention times and analyzed using a continuous flow analyte specific GC-IRMS (thermoscientific 1310 trace Gas chromatogram with a thermoscientific Delta Q isotope ratio mass spectrometer). The GC-IRMS reference gas was calibrated to the Vienna Pee Dee Belemnite scale using secondary reference materials. Preliminary results from the analysis of NIST SRM 1991, a mixed petroleum and coal tar creosote standard, and a collected sample from creosote-impacted surface water in Northern Oregon near the Portland Harbor Superfund site show respective isotopic signatures within 1% of each other, indicating that data collected via passive sampling will return a ratio, measured by delta signatures, consistent with expected literature values for that source. Further analyses of samples from different PAH sources returns similar results, with analyzed samples including natural gas fracking sites, tobacco smoke, and the Portland Harbor Superfund site in Portland, OR. When comparisons of these point sources occur, it becomes clear that the unique combinations of detected PAHs and their delta signatures is necessary to develop a fingerprint of each source. Future sample collection and application of this method will investigate the use of isotopic mixing models to pull apart source contribution fingerprints in these samplers.

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3.09.A.T-04 Identifying Contaminant Fingerprints and Endpoints-Specific Risk Drivers Pattern in Source-Related Effluents using Non-Target Screening

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Source-related effluents introduce complex micropollutant mixtures into the environment. Traditional targeted monitoring captures only a fraction of these contaminants, while effect-based methods integrate biological impacts of both known and unknown compounds. Non-target screening (NTS) provides comprehensive chemical coverage but generates vast datasets requiring intelligent prioritization. This study developed a biologically guided computational workflow to identify source-specific chemical fingerprints and endpoint-specific effect drivers in effluents.

We analyzed 104 water samples from industrial and environmental sources in seven European countries. Samples underwent solid-phase extraction, LC-HRMS analysis, and testing in nine in vitro bioassays covering hormonal activity, xenobiotic receptor activation, mutagenicity, oxidative stress, PFAS-related activity, and neurotoxicity. Chemical profiling detected 154,572 features, with structural annotation performed using SIRIUS and CSI:FingerID.

To prioritize biologically relevant features, we applied MLin vitroTox predictions to estimate activation probabilities across eight bioassays for every detected feature. Features exceeding bioactivity thresholds were retained as potential risk-driver candidates, reducing the feature space while preserving toxicological relevance. Random Forest classifiers trained with leave-one-out cross-validation identified source-specific contaminants within these filtered subsets, successfully differentiating effluent groups. LASSO regression with five-fold cross-validation then linked feature intensities to measured bioassay responses, identifying statistically supported effect drivers.

The workflow generated two complementary outputs: source-specific chemical fingerprints from Random Forest classification and endpoint-specific effect-driver fingerprints from LASSO regression. Strong structure-effect relationships emerged for androgenicity, where prioritized features predominantly matched steroid and steroid-like structures including testosterone derivatives, hydroxyprogesterone, and methylated androstanes. Oxidative stress responses showed weaker correlations, reflecting non-specific pathway activation, with O-glycosides among selected features.

This integrated approach demonstrates how predicted toxicity, source identity, and measured biological effects

can be systematically connected through computational prioritization of NTS data for environmental risk assessment.

3.09.A.T-05 Sustainable green roofs for the implementation of the sponge city: Determination of ecotoxicologically safe construction products for green roofs

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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 urban heat islands through evaporative cooling and reduces drought.

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 the further course, a model roof is constructed on a reduced scale with evaluated building products and the retained and discharged rainwater is examined with regard to its ecotoxic effects. The aim is to define building materials that can be used safely for the sponge city concept for green roofs.

To identify such environmentally safe products, targeted ecotoxicological and analytical testing is conducted on a selection of 20 construction products. 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 (DSLTT, as per DIN EN 16637-2 is used, while granular products are assessed with the upflow percolation test (DIN EN 16637-3). The analysis of the eluates is now largely finalized. Complementary characterization was performed using various analytical methods to ensure thorough evaluation of the eluate composition.

Disclaimer/Disclosure:

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3.09.B Environmental Forensics: Tracking Sources and Evaluating the Effects of Chemicals in Urban and Other Environments

3.09.B.T-01 Temporal Impact from PFAS Source Identification to Downstream Response- Lessons Learned and Implications

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One of the primary hurdles in PFAS investigations is distinguishing between overlapping sources, such as industrial and domestic discharges, atmospheric deposition, and legacy contamination from firefighting foam applications. These sources often result in complex mixtures of PFAS, including parent compounds, transformation products, and precursors, making it difficult to link contamination to specific activities or origins. This presentation aims to use a multi-year PFAS monitoring site combining with a multi-tiered approach to identify the pros, cons and limitations on PFAS forensics applied today and propose the next generation of PFAS forensic solution. Most publications on PFAS fingerprinting or forensic studies are limited to small or single sampling event at downstream receptor site/facility. Temporal datasets to observe migration and transformation over time are often recognized as data gaps. While large-scale PFAS forensic study is of interest, for a detailed assessment, this project chose a PFAS investigation site impacted by a historical fire event, paper industry and background sources. Groundwater samples have been collected across the site and also from background, upgradient and sidegradient locations since 2018. The multi-year datasets illustrated the measured PFAS compositions and concentrations changes with time and use clustering analysis to assess how the data

clusters evolve and could impact the source identification and their subsequent fate and transport. The data clusters further were used to interpret precursor transformation and PFAS sources through integration with biotransformation and source databases. Data gaps and future advancement on digital tool are identified through this project. The composition and cluster analysis are determined useful. Better streamlining of “origin” signatures to the downstream PFAS monitoring data remain challenging even when cluster analysis provides value. The nontarget analysis and TOP assay are expected to add additional lines of evidence to enhance PFAS forensics.

3.09.B.T-02 Non-target Screening and Physicochemical Profiling of Infiltrated Chemicals in Stormwater Biofilters for groundwater recharge

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Urban areas with dense populations produce high pollutant loads due to intensified human activity and the prevalence of impermeable surfaces. These conditions enhance runoff and contaminant transport, as natural barriers such as vegetation and soil are largely absent. To address this, Sustainable Urban Drainage Systems (SUDS) have been introduced with the aim of reducing impervious surfaces, decentralizing runoff management, and alleviating pressure on centralized treatment facilities. Research indicates that while SUDS can effectively retain hydrophobic compounds, more persistent and mobile pollutants often bypass these systems without removal. Although the octanol–water partition coefficient (log K or log D) has traditionally been employed to assess pollutant mobility under frameworks such as REACH, recent studies are investigating alternative molecular descriptors that may better capture contaminant elution in soil environments. Given that SUDS effluents can infiltrate groundwater—recognized by the European Union as a strategic resource requiring protection—it is essential to evaluate their performance against the diverse pollutants present in urban runoff. Previous investigations have already identified highly polar compounds in groundwater that were also detected in runoff samples.

In this context, High-Resolution Mass Spectrometry combined with Non-Targeted Analysis (HRMS-NTA) was applied to detect multiple classes of compounds at trace concentrations. The study compared water samples collected before and after SUDS treatment, categorizing pollutants as retained, transmitted, or newly formed/transformed within the system. Ultimately, screening based on physicochemical properties, using both conventional and chemoinformatic approaches, enabled the identification of mobile compounds capable of percolation. Principal component analysis (PC1–PC3) provided a multidimensional representation of physicochemical diversity across the dataset. PC1 captured molecular size and flexibility, PC2 reflected structural complexity and aromatic–polar character, and PC3 described reflected the balance between lipophilicity and polarity. The observed trends suggest that the biofilter preferentially allows the passage of moderately polar and aromatic compounds containing heteroatoms or hydrogen-bonding functionalities, while more hydrophobic or rigid species are comparatively less represented.

Disclaimer/Disclosure:

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3.09.B.T-03 POPs in Brown Waters - Revealing Spatial Patterns of PCDD/Fs, PCBs and PBDEs in Fish along Gradients in the Northern Baltic Sea

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High concentrations of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polybrominated diphenyl ethers (PBDEs) have been found in the Baltic Sea for decades. Their lipophilic properties have resulted in elevated concentrations in fatty fish such as herring and salmon. Bioavailability of pollutants are affected by the quality of dissolved organic matter (DOM) and organic carbon is an important parameter in the cycling and

accumulation of POPs. The hypothesis is that differences in bioaccumulation patterns in fish can be observed and follow the carbon-quality gradient in the Baltic Sea. The model fish used is the three-spined stickleback (*Gasterosteus aculeatus*) having high fat content as well as a migrations pattern moving from coast to offshore and back during their lifecycle.

Sticklebacks were collected in defined bays along the Swedish and Finnish coasts as well as Offshore in the Bothnian Sea and Bothnian Bay (n=23). The fish samples were extracted, purified and target analytes were determined by HRGC-HRMS. Concentrations and congener composition of PCDDs, PCDFs, non-ortho-PCBs, indicator-PCB (I-PCB), mono-ortho PCB, and PBDE were compared and assessed by principal component analysis (PCA), across three station categories: Sweden, Finland and Offshore, for a spatial overview of concentrations.

Concentrations for PCDDs differ significantly between all three station categories ($p < 0.01$); PCDF differ significantly between Finland- Offshore stations ($p < 0.01$); PBDE differ significantly between Sweden-Finland ($p < 0.001$), and Finland- Offshore ($p < 0.001$). Though no significant differences could be seen for I-PCBs, mono-ortho PCBs or non-ortho PCBs on a larger scale, there are differences between specific stations which are more impacted by anthropogenic sources. This study suggests that the inflow of DOM from allochthonous sources via river inputs in the different coastal areas is an important regulator of the levels and composition of POPs in northern brown waters.

3.09.B.T-04 Biomineralized shells as forensic bioarchives: metal(loid) mixtures and nanoparticles from metallurgical particulate matter accumulate and persist in marine bivalves

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Metallurgical industries release settleable particulate matter (SePM) enriched with metal(loid)s and metallic nanoparticles (NPs), which deposit into coastal waters and can interact with biomineralized structures. We evaluated two ecologically and economically important bivalves, the mussel *Perna perna* and the oyster *Crassostrea rhizophorae*, to determine how SePM-derived contaminants are incorporated and retained in their shells. Organisms were exposed for 30 days to increasing SePM concentrations (0.01, 0.1 and 1.0 g L⁻¹) and then maintained for 30 days in clean seawater to assess depuration. Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy revealed metallic particles and nanoscale aggregates embedded within calcite and aragonite layers of both species. Inductively coupled plasma mass spectrometry confirmed concentration-dependent accumulation of multiple elements, including Al, Ti, Mn, Fe, Zn, Sr, Ba, La and Ce. Although depuration reduced some element concentrations, neither species returned to baseline levels, indicating limited reversibility once contaminants are incorporated into the shell matrix. Mechanical compression testing showed that exposure to SePM reduced shell strength in both species, with *C. rhizophorae* exhibiting greater structural loss, consistent with its higher particulate retention. *P. perna* displayed faster incorporation dynamics, while *C. rhizophorae* retained a broader and more persistent elemental signature. Overall, the combined chemical, microstructural and mechanical evidence demonstrates that mussels and oysters preserve stable and source-specific particulate fingerprints derived from metallurgical emissions. These findings highlight both species as complementary forensic bioarchives for source attribution and long-term monitoring of particulate pollution in coastal ecosystems.

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3.09.B.T-05 Occurrence of 7 bisphenols in surface soil and sewage sludge from Trondheim, Norway

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Bisphenols (BPs) are a series of analogues that are extensively used in the production of plastics and as additives for the manufacturing of other materials. Over years they have been detected in diverse environmental matrices like soil, biosolid etc. and exposed to human and other biota, causing both environmental and health concerns. Trondheim, the fourth largest city in Norway, has imported treated sewage sludge from local wastewater treatment plants (WWTPs) for landscaping purpose, but the levels of BPs in Trondheim soil remain largely unknown. This study collected surface soil in urban environments and sewage sludge from 2 WWTP in Trondheim, Norway from 2020 to 2024. Following solid phase extraction, BPs were analysed by ultra-performance liquid chromatography-tandem mass spectrometry. The study aims to (1) discover the BPs level in the surface soil in Trondheim, and (2) determine if local WWTPs are a source of BPs occurrence in Trondheim soil. The results unravelled alarmingly high concentration of BPs in sewage sludge in WWTPs in Trondheim, which are comparable to that found in identical type of samples in other countries. This finding underlines that WWTPs serve as a sink for contaminants of emerging concern. Besides, BPs concentration in Trondheim surface soil was lower than those found in soil internationally, and statistical test has revealed the possible land application of treated sewage sludge was not the major pollution source of BPs in Trondheim surface soil. Consequently, further research is required to investigate the sources of bisphenol pollution in the urban soil of Trondheim.

Disclaimer/Disclosure:

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3.09.P Environmental Forensics: Tracking Sources and Evaluating the Effects of Chemicals in Urban and Other Environments

3.09.P-Tu219 Searching for unknown PFAS sources, a comparison between domestic and industrial wastewater in the Netherlands.

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People and the environment come into contact with PFAS in various ways. Prolonged exposure to these chemicals can be harmful to health of people and the environment. To reduce this exposure, it is important to know the sources that release these substances. We know that PFAS end up in wastewater through households, for example through cleaning agents, cosmetics and washing of clothing. On the other hand, we also know that PFAS may come from industrial sources and ultimately end up in wastewater through discharges. Not much is known about PFAS from smaller industrial sources. This was reason for RIVM to study whether measurements at wastewater treatment plants (WWTP) would provide insight into unknown industrial and other sources. This turns out to be the case. The measurements revealed sources that cannot be explained by PFAS in domestic wastewater. Untreated wastewater (influent) entering a WWTP is always a mixture of domestic and industrial wastewater. The ratio of these two types of wastewater differs in each treatment. RIVM studied the influent of two groups of WWTPs in the Netherlands. The first group consisted of 11 that mainly treat domestic wastewater. The second group consisted of 6 WWTPs that treat a large amount of industrial wastewater, with possible, unknown sources of PFAS. For the first group of WWTPs, the influent was found to contain the same quantities of the same PFAS compounds. This 'pattern' can also be found in drinking water, meaning that part of these PFAS end up in the wastewater through the use of tap water. These analyses must take into account the source of the drinking water (surface water, groundwater). This is because drinking water sourced from surface water contains more PFAS than drinking water sourced from groundwater. The same pattern of PFAS compounds was also found at the WWTPs that treat industrial wastewater. However, more PFAS were found at some WWTPs than expected based on the number of local residents. This is an indication that industrial sources of PFAS yet unknown are present. Further research is needed to determine these sources.

3.09.P-Tu220 Microplastic Contamination Pathways in The Semi-Enclosed Freshwater Basin of Lake Como, Italy

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Global plastic production continues to rise, and microplastics (MPs) are now widespread across environmental compartments, largely originating from land-based sources such as urban runoff, agriculture, wastewater treatment plants (WWTPs), and untreated discharges. To date, the contribution of untreated discharges during storm-related events, together with road washoff, remains poorly explored, considering that climate change is expected to increase the frequency of such events. Due to its semi-closed shape and long hydraulic residence time, Lake Como represents a particular case for evaluating how episodic untreated discharges and continuous treated effluent inputs influence contaminant dynamics. Therefore, the aim of this study is to analyze the concentration and investigate the source of MPs, as well as their fate in the semi-enclosed lake systems and to support the development of mitigation strategies. MPs were sampled from River Breggia, and from River Cosia in different hydraulic conditions, from input and output water of the Como WWTP, while lake water was sampled in stratified (summer conditions) for epilimnion and hypolimnion. Lake sediment was also sampled. MPs were extracted via oxidation with 30% H₂O₂ and density separation using ZnCl₂ (1.8 g/cm³). MPs were identified through visual observation under fluorescence microscopy, after Nile Red staining, and their polymer composition and mass were determined using Pyrolysis–Gas Chromatography–Mass Spectrometry (Py–GC–MS). Results show that storm events sharply increased MP loads in the river. In Lake Como, stratification and circulation led to very low pelagic concentrations but strong accumulation in littoral zones and sediments. Fine particles (<50 µm) and fragments dominated across matrices. PE and PP were the main polymers in waters, with SBR prominent in stormwater, while sediments and hypolimnetic samples showed greater diversity, mainly PE, PP, and N66. These findings highlight the vulnerability of semi-enclosed lakes to episodic untreated discharges and continuous effluent inputs, underscoring the need for integrated management strategies.

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3.09.P-Tu221 A Systematic Comparison of Cryogenically Milled Tyre Tread (CMTT) and Tyre and Road Wear Particles (TRWP) and the Implications of Using CMTT for Environmental Studies.

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Tyre and road-wear particles (TRWP) in the environment is receiving increasing attention due to their substantial generation (ca. 1.3 mil t a⁻¹ in EU), their diffuse release into the environment, and growing awareness of the toxicity of the compounds which leach from them. TRWP is challenging to acquire, therefore studies often use cryogenically milled tyre tread (CMTT) as a proxy, though their differences are not fully understood. This study directly compares CMTT and TRWP produced from the same 3 tyres, analysing their chemical extracts and leachates to evaluate differences in composition and the suitability of CMTT for laboratory leaching studies.

Repeated extractions and leaching were performed on both the CMTT and TRWP. Leachables and extractables were analysed by UPLC-HRMS, with target screening of 45 tyre related compounds of which 35 compounds were quantified in the extracts and 31 in the leachates. This was then complemented by a suspect- and non-target screening.

Overall, CMTT extracts contained three times higher total concentrations of target compounds than TRWP extracts. The antioxidant 6PPD dominated CMTT extracts (71%) but contributed only 11% in TRWP, with its TRWP concentration only 5% that of CMTT. Other p-phenylenediamines (DTPD, IPPD) were reduced in the TRWP. Transformation products such as 6PPD-Q and N-formyl-6PPD were enriched in TRWP, though not enough to account for the full loss of parent compounds, indicating formation of additional unmonitored transformation products. Suspect screening also suggested increased oxidative DPG transformation products in TRWP. Overall, CMTT contained more precursor compounds while TRWP was richer in transformation products, reflecting composition changes during abrasion. Leachates showed similar trends: TRWP leachates were more dominated by transformation products than leachates of CMTT. The presence of unquantified transformation products in TRWP and its leachates raises concerns about their environmental release and unknown ecological effects.

In conclusion, using CMTT as a TRWP proxy could lead to an overestimation of parent compound concentrations and underestimation of transformation products, potentially misrepresenting environmental impact. This has significant implications for chemical monitoring and toxicity studies reliant on CMTT.

3.09.P-Tu222 Evaluation of the Spatial Distribution of Microplastics Mass Concentrations in Air at a Regional Level by Means of Tree Leaves as Passive Samplers and PY-GC-MS

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Microplastics (MPs) are emerging contaminants of global concern. Recent studies show that MPs can accumulate on plant surfaces, including leaves, and enter the atmosphere through wind, dust resuspension, and urban emissions.

Moreover, rainfall can wash these particles off from leaves into terrestrial ecosystems, potentially affecting soil quality and contributing to the load of MPs in aquatic ecosystems by deposition. So far, data of MPs on leaves as well as their source, transport and behaviour are still poorly explored. The aim of this study was to evaluate the spatial distribution of MPs deposition on leaves in different selected areas at a regional level in Italy. MPs in leaf samples were characterized by Pyrolysis - Gas Chromatography and Mass Spectrometry (Py-GC-MS) for twelve polymers. The preliminary results showed a general similar trend for most polymers at a regional scale with peaks from several polymers in areas affected by intense traffic, compared to lower polluted areas (e.g., low traffic zones, green parks, forest). Most common polymer found were PET, PE, N66, PP, PS, N6, SBR, and PVC. MPs correlated with PM concentrations measured on leaves, showing peaks in high PM affected areas.

3.09.P-Tu223 Environmental exposure to tire-related pollutants in outdoor artificial turf sport centers

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Tire-related pollution has emerged as a significant threat to the short- and long-term sustainability of aquatic ecosystems, particularly those exposed to stormwater or street runoff. Although current research largely focuses on well-known pollution hotspots, less common sources should not be overlooked. In Europe, 52% of end-of-life tires are recycled into granulated rubber tire particles (RTP), which are commonly incorporated into cement and used in the manufacture of artificial turf. RTP is also frequently applied as infill on the surface of artificial turf sports fields to enhance grip, potentially turning these facilities into underrecognized hotspots for tire-derived pollutants. Concerns have already been raised regarding human health risks, such as exposure to carcinogenic substances in recycled rubber and increased incidences of leukemia and lymphoma among athletes; yet environmental exposure pathways remain comparatively understudied.

In this work, we investigate the presence of tire-related organic micropollutants in snow collected from outdoor artificial turf sports centers, assessing their potential to release pollutants through short-term leaching. To complement the field study, we conducted accelerated leaching experiments on RTP to evaluate their long-term contribution to aquatic contamination. Four outdoor artificial turf sports centers in the municipality of Uppsala served as case studies.

Snow samples were processed via solid-phase extraction, while RTP underwent solid-liquid extraction. All extracts were analyzed using liquid chromatography coupled to high-resolution mass spectrometry, screening for more than 130 tire-related organic micropollutants. Several compounds, including benzothiazole derivatives, urea derivatives, phthalates, benzotriazole derivatives, and 6PPD-quinone, were detected in both meltwater and RTP extracts. These findings reveal a complex mixture of tire-derived pollutants entering the environment from a previously underappreciated source.

Overall, our results highlight the need for further research into effective mitigation strategies for tire-related pollution and underscore the importance of developing alternative infill materials that minimize environmental impact while supporting sustainable urban development.

3.09.P-Tu224 Monitoring and Speciation of Road Dust Contaminants in a Perialpine French Lake

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Tire Wear Particles (TWPs), a major component of road dust (RD), pose a significant threat to aquatic ecosystems. Abrasion of tires on roads generates microplastic-like particles that are transported to freshwater ecosystems during stormwater runoff. These particles release multiple pollutants, including metals such as zinc (Zn) and copper (Cu), polycyclic aromatic hydrocarbons (PAHs), and organic additives like the diphenylguanidine (DPG) or 6PPD and its oxidation product 6PPD-Q. Automotive sources are estimated to contribute nearly 30 tons of TWPs per year to Lake Geneva (Lac Léman, Switzerland). Despite increasing concern regarding the ecotoxicity of these compounds, their environmental fate and speciation in lacustrine systems remain poorly documented. This research project aims to investigate the transport pathways of RD and

TWPs in freshwater ecosystems during different meteorological events and to characterize the speciation of their associated contaminants. Over an 18-month monitoring period, we will quantify the TWP and several of their associated contaminants: Zn, PAHs, DPG, 6PPD, and 6PPD-Q at both a contaminated and a reference site. The contaminated site will be located near a spillway on the shore of Lake Annecy, a highly anthropized littoral zone subject to dense road traffic. To assess speciation, contaminants will be measured across multiple environmental compartments, including runoff water, the dissolved phase of the water column, suspended organic matter, macroplankton, and lake sediments near the spillway. By combining long-term monitoring with a multi-compartment analytical approach, this study will provide new insights into the transfer dynamics of RD and TWPs within lacustrine ecosystems. The project will improve understanding of contamination processes in the littoral zone at the crucial interface between freshwater ecosystem and anthropogenic activity.

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3.09.P-Tu225 Microbial Community Adaptation Enhances Biodegradation of Tire-Wear-Derived Contaminants in Urban Roadside Soils

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Tire and road wear particles (TRWPs) are a major source of diffuse urban pollution, continuously released from vehicles and accumulating in urban roadside soils. These soils represent both a sink and an active biogeochemical environment where contaminants may undergo transformation. In this study, we investigated the biodegradation potential of the 5 typical TRWP-derived organic contaminants 1,3-diphenylguanidine (DPG), dicyclohexylamine (DCH), hexamethoxymethylmelamine (HMMM), 6PPD-quinone (6PPDQ), and 2-mercaptobenzothiazole (2-MBT), in urban roadside soils collected along a traffic intensity gradient in Leipzig, Germany.

Batch biodegradation experiments demonstrated consistent traffic-dependent trends: soils exposed to high-traffic exhibited substantially faster and more complete degradation of the model contaminants. Non-target analysis revealed a wider variety of transformation products, appearing earlier and at higher abundance in high-traffic soils, indicating enhanced or more diverse metabolic activity. These patterns suggest that long-term exposure to traffic-derived contaminants may drive microbial community adaptation, resulting in increased enzymatic capacity for contaminant transformation.

Our findings highlight roadside soils as dynamic biogeochemical environments with significant natural attenuation capacity. Understanding how chronic exposure shapes microbial function is essential for predicting contaminant fate in urban environments and for designing nature-based stormwater treatment systems, such as tree trenches and biochar-amended filters, that harness or enhance these microbial processes.

3.09.P-Tu226 A multi-tier ecotoxicological assessment : strengthening waterbodies evaluation through in vivo, in vitro and in situ ecotoxicity tools.

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The 2024 ecotoxicity surveillance network was carried out in 33 sampling locations of the Walloon WFD monitoring programme (Escaut, Meuse and Rhine basins). Its purpose is to assess how anthropogenic pressures impacted waterbodies by combining in vivo and in vitro bioassays with in situ biomarker responses in caged organisms. This five-year monitoring cycle follows the surveys conducted in 2004, 2009, 2014 and 2019. To address the need for a more integrative evaluation of toxic pressures, the programme combines acute and chronic in vivo assays (*Vibrio fischeri* (bacteria), *Pseudokirchneriella subcapitata* (algae), *Brachionus calyciflorus* (rotifer)), in vitro tests targeting endocrine and genotoxic activities (YES, YAS, anti-YES, anti-YAS, and UMU tests), and biomarker measurements in caged *Gammarus* spp. (acetylcholinesterase (AChE) inhibition, glutathione S-transferase (GST) induction, feeding rate). These biological tools complement standard physicochemical monitoring and help identify modes of action and potential long-term risks. Results show contrasted trends. Acute bacterial toxicity decreased further compared with 2019, while rotifer and algal sensitivity increased at several sites, suggesting potential emerging pressures. In vitro assays revealed widespread estrogenic activity, detected in over 90% of samples, with two-thirds of stations exceeding the

proposed EQS for 17 β -estradiol. Genotoxic responses were observed in one-third of sites. Biomarker responses in gammarus highlighted severe organism stress at several locations, including high mortality, significant AChE inhibition pointing to insecticide exposure, marked GST induction and reduced feeding activity. For each of the 33 investigated stations, a radar chart summarizes the overall ecotoxicological quality based on the results obtained from the different bioassays. Overall, the integration of multiple biological levels provides a refined understanding of toxic impacts. Stations in good chemical and ecological status generally showed low ecotoxicity, while degraded water bodies displayed consistent effects across assay types. The combined approach strengthens the assessment of ecological status and supports early detection of emerging toxic risks, guiding water-management strategies.

3.09.P-Tu227 Microbial fingerprints as forensic tools for unraveling chronic versus acute agrochemical exposure in agricultural soils

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Intensive agriculture introduces complex chemical mixtures into the environment, creating a pollution gradient ranging from diffuse application to acute point sources. A major challenge in environmental forensics is to distinguish, retrospectively, between the origins of such contamination, in order to tailor effective remediation strategies. This study evaluates the environmental footprint of five recalcitrant agrochemicals (atrazine, acetochlor, metolachlor, azoxystrobin and 2,4-dichlorophenoxyacetic acid) across three distinct agroecosystems in Argentina. To unravel the effects of management intensity, we utilized a stratified design comparing four scenarios per site: pristine soils, minimal intervention strategies, conventional management and machinery washing sites (acute contamination).

Our approach integrates the isolation of native degraders with high-throughput 16S rRNA and ITS2 amplicon sequencing. Restrictive physiological assays using agrochemicals as a nitrogen source successfully isolated tolerant and degrader strains, including *Rhodococcus* sp. and *Acinetobacter baumannii*, which demonstrated high efficacy (>90%) in degrading metolachlor and azoxystrobin, confirming the site-specific potential for natural attenuation.

Regarding community dynamics, we hypothesize that the distinct selective pressures of chronic versus acute exposure create unique molecular fingerprints within the microbiome. This retrospective approach is crucial in forensic contexts where chemical residues may no longer be detectable by conventional analytics due to environmental fate processes (e.g., sorption or biodegradation), yet the historical impact remains imprinted in the microbial community.

Ongoing metabarcoding analysis aims to identify specific microbial taxa that serve as differential biomarkers for each scenario. By correlating community structure shifts with chemical history, this research provides a semi-quantitative framework to track pollution sources, supporting both forensic investigations and the development of targeted bioaugmentation approaches to restore soil health.

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3.09.P-Tu228 Presence and Fate of Chemicals associated with Biosolid Application to Land to Groundwater: Current Evidence and Knowledge Gaps

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Biosolids produced from wastewater treatment works (WwTWs) are applied to land as a nutrient rich soil amendment in many countries. However, the range and concentrations of contaminants found in biosolids, and underlying soil and groundwater remains poorly characterised, outside of a few well studied chemical groups. A

systematic literature review was conducted to identify and collect available data for chemicals and microplastics (MPs) present in WwTW biosolids, amended soils and underlying groundwater. Our aim was to establish their status in countries in the UK/Western Europe with developed wastewater infrastructure. Hence, data collection was focussed on these regions, except where data was limited when all available information was considered. Data of approximately 14,000 chemical or MP measurements in biosolids and biosolid-amended soils were extracted, with measurements for 786 contaminants for biosolids and 21 different contaminants for biosolid-amended soils. In soil porewaters, leachate and groundwaters, 879 measurements for 80 contaminants were retrieved. Analysis of this data showed that a broad spectrum of chemicals has been detected and measured in biosolids, with median exposure concentrations in the order of nutrients > MPs > metals/metalloids > personal care products (PCPs) > plasticisers > pesticides > pharmaceuticals > industrial solvents > persistent organic pollutants. For biosolids-amended soils and underlying groundwaters fewer studies of the associated chemicals are available, with nutrients and metals/metalloids being the major focus of research to date. Thus, there is a clear gap in the knowledge of what contaminants in biosolids are most likely to persist in treated soils and transfer to groundwater.

This review gives a first comprehensive overview of contaminants present in biosolids and the soils and groundwater below their application. Our review highlights the important need for integrated studies which examine biosolids, soils, unsaturated and saturated zones, and the expansion of monitoring efforts beyond metals to include PCP, PFAS, pharmaceuticals, plasticisers and MPs. A greater understanding of bioavailability and exposure potential, via the application of field studies, is needed to assess chemical fate and exposure in soils and groundwater, and associated risk of these WwTW biosolids derived contaminants.

3.09.P-Tu229 Tracking chromium sources in a water supply reservoir in Cape Breton, Nova Scotia, Canada: Implications for watershed and reservoir management

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Sludge produced as a waste product of water treatment at MacAskill Reservoir, Cape Breton, Canada contains elevated Cr, which is an environmental concern and a significant treatment expense. Legacy coal mining and surface processing are potential point sources of Cr. Till that incorporates coal-bearing strata and atmospheric Cr from coal power generation are also potential Cr sources. We employ a variety of investigative techniques to track the source(s) of Cr to effectively manage the watershed to minimize Cr in the water treatment processes. Cr in watershed groundwater and surface water samples was below analytical detection limits.

Paleolimnological data from a nearby reference lake recorded atmospheric pollutant impacts (Pb) but did not indicate atmospheric sources of Cr. Chromium was most prevalent in watershed soils which contained > 70 ppm Cr likely derived from Cr-bearing minerals in nearby Proterozoic bedrock; tailings from a legacy mine contained > 440 mg/kg Cr. Reservoir bottom sediment cores averaged 40 ppm Cr. Our data suggests that Cr is transferred into the reservoir as either fine-grained sediment or as stable Cr(III) that is released under reducing conditions through the interaction of till and underlying bedrock with acidic surface and groundwater. These data suggest Cr concentrations at the water treatment intake can be lowered by limiting landscape disturbances, particularly in wetlands within the watershed. Disturbance of legacy coal workings also has significant potential to release Cr into water systems and requires further assessment and monitoring to better quantify risk.

3.09.P-Tu230 Unveiling Hidden PFAS: Thermal Transformation of PVDF Binders During Lithium-ion Battery Recycling

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The global transition toward renewable energy has accelerated the demand for lithium-ion batteries (LIBs), which are essential for energy storage, electric vehicles, and portable electronics. Battery recycling has become a strategic priority to recover critical materials and minimize wastes. The presence of fluorinated binders, particularly poly(vinylidene fluoride) (PVDF) currently used to make battery electrodes, poses significant operational and potential environmental challenges. PVDF binder offers exceptional chemical stability but complicates its thermal removal during battery recycling, potentially generating toxic fluorinated emissions. Despite the widely used pyrometallurgical battery recycling processes, the thermal decomposition and emission profiles of the PVDF binder within spent LIBs metrics remain poorly understood. We investigated the thermal degradation of PVDF and cathode materials under representative recycling conditions. Our results revealed the generation of a diverse spectrum of fluorinated volatile, condensed, and solid-phase products, whose composition varied with temperature, oxygen availability, and cathode chemistries. We identified key temperature thresholds associated with LIBs material breakdown and the subsequent formation of fluorinated alkanes, aromatic substances, and oxygenated hydrocarbons. The findings advance the understanding of per- and polyfluoroalkyl substance (PFAS) behavior during LIBs recycling and highlight the critical needs to integrate PFAS management into sustainable battery recycling strategies.

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3.09.P-Tu231 Heavy Metal Contamination in Urban Aerosols of Dhaka: Implications for Air Quality

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Sixteen (16) TSP (Total Suspended Particles) aerosol samples were collected from the rooftop of a four storied residential building of Dhaka Polytechnic Institute senior staff quarters, which is situated in Teajgaon, Dhaka. TSP samples were collected on pre-weighted glass fiber filters using a high-volume air sampler (APM 860) having flow rate 1.4 m³/min. in January, February, May and June of 2021 once a week basis and were analyzed for heavy metals including Pb, Zn, Cd, Fe, Cu, Ni and Mn using atomic absorption spectrophotometer (models: Spectra AA220 and AA240FS, Varian, Australia) equipped with electro-thermal temperature controller and flame. Abundance of heavy metals in Dhaka urban aerosols showed a distribution patterns of Zn>Fe>Mn>Pb>Cd>Cu>Ni. Concentration of Cd in Dhaka urban aerosols showed ~12 times higher concentration than that of uncovered in aerosol samples collected from Philippines and is ~13 times higher than that of Iranian aerosol. However, concentration of Ni is 327 times lower than that of measured in aerosol samples collected from Philippines, but which is 3 times higher than of Iranian urban aerosols. Enrichment factor analysis indicated that Pb, Zn, Cd, Cu and Fe are mainly emitting from anthropogenic sources. Carcinogenic risk from Ni and Cd towards different demographic level of the study area was almost same. Where we found that out of every 1000 people, on an average 14 children, males and females are at cancer risk from Dhaka urban aerosols. However, non-cancer risk induced from Zn, Cu and Mn is more significant for the children followed by adult male and female. The risk levels for the non-carcinogenic heavy metals occurred in the following order: Cu > Zn > Pb > Mn. Non-carcinogenic metals can cause harm to children more easily than those of men and women. Positive Matrix Factorization (PMF) analysis identified four major sources of these heavy metals in Dhaka urban aerosols, which includes road dust resuspension, vehicular emissions, industrial metal processing, and mixed traffic-combustion.

3.09.P-Tu232 Effects of different climatic conditions on metals from soils collected from waste disposal sites

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Metal contamination in landfill sites poses significant environmental and public health risks. This research investigated the concentration trends of eight metals (Co, Zn, As, Sr, Ag, Cd, Sb and Pb) in soils from both legal landfills and illegal dumping locations, considering various temperature (20°C, 22°C, 25°C) and moisture conditions (30%, 50%, 60% of water-holding capacity) to replicate climatic fluctuations when exposing earthworms (*Eisenia andrei*) to the various soils. The findings indicate that both site management practices and environmental factors significantly influence the bioaccumulation of metals by earthworms in landfill settings. Illegal dumping sites pose a significantly higher risk to soil health, as earthworms accumulate alarmingly high levels of several metals. The conditions of temperature and moisture have a profound impact on the bioavailability and uptake of metals, with cooler temperatures (20°C) and lower moisture levels (30%) typically facilitating higher accumulation.

This research emphasises the environmental hazards associated with poorly managed waste disposal sites and stresses the necessity of incorporating climatic variables into ecotoxicological evaluations. The findings highlight the urgent requirement for adaptive waste management strategies to respond to climate-induced changes in contaminant behaviour. Employing standardised bioassays and *E. andrei* as a responsive bioindicator offers substantial support for regulatory measures designed to alleviate the effects of soil contamination.

3.09.P-Tu233 Assessing Metal Contamination and Ecological Risk in Vineyard Soils of Western Cape, South Africa

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Soil contamination by metals has become a global concern, driven by increasing awareness of risks to food safety. This study investigates the sources and spatial distribution of metals in vineyard soils (VS) across the

Western Cape, South Africa. Soil samples were collected from six vineyard farm sites (n = 42 per site; total n = 252; row and inter-row samples) for ecological risk identification. Metal concentrations were measured using a Niton XL3t GOLD portable XRF following USEPA Method 6200. Across the vineyard sites, 33 metals were detected. Mean concentrations (mg/kg) varied widely, with notable ranges for Ag (3.43–3.62), As (1.98–3.17), Ba (23.26–35.68), Cd (5.17–5.48), Cr (6.43–21.98), Cu (8.52–27.93), Fe (1464.73–16632.83), Hg (3.95–4.38), Mn (31.94–135.50), Ni (15.10–18.66), Pb (2.64–4.04), V (26.15–123.70), and Zn (8.41–33.60). When compared with FAO guideline limits, Cd, Hg, and V exceeded their respective thresholds (1.0, 0.50, and 50 mg/kg, respectively). These exceedances indicate potential risks to biota and food safety, suggesting that some sites may pose ecological and human health concerns. Ecological risk was further evaluated using the geoaccumulation index (Igeo) and pollution index (PI), based on background concentrations from pristine university garden soils as well as published values from South Africa, Canada, and the Netherlands. All vineyard sites showed low to moderate anthropogenic enrichment for the measured metals, with no consistent multi-metal pollution pattern when compared with garden soil background levels. However, when compared with background values from the Netherlands, vineyard soils showed higher pollution for Cd, V, and Hg (Igeo 1–3.3; PI 1.1–14.6). Background data from South Africa also indicated moderate to high risk for As, Cd, and Hg (Igeo 1.3–4.2; PI 1.1–27.4). Canadian reference values highlighted moderate to high contamination for Cd and V (Igeo: 4.5–4.6; PI 1.2–36.5). Overall, the vineyard soils fall within low to high contamination ranges for several metals, particularly As, Cd, Hg, and V. These findings reveal clear variations in metal loading across the region and pinpoint sites that may require closer ecological and food safety monitoring.

3.09.P-Tu234 Distribution of Potentially Toxic Elements in Sediments in Select Rivers and Creeks in Calgary, Canada

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Watersheds in Calgary, a metropolitan city in Alberta, contain mixes of varying land use including parks, residential, commercial, industrial, institutional, and agricultural. Major watersheds including Bow River, Elbow River, Fish Creek, Nose Creek, Pine Creek and the Shepard Wetland. The Bow River and Elbow River provide drinking water for the city while along with the other creeks also provide valuable habitat for fish and animal species and spaces for recreation. Surface runoff which typically empties into these watersheds can potentially introduce pollutants including various potentially toxic elements (PTEs) into sediments. Thirty-five sediment samples were collected from rivers and creeks in Calgary to assess how the surrounding land use influence PTEs distribution in the sediments. Total PTE concentrations were analyzed by x-ray fluorescence and the physicochemical properties of surface water were also determined. The mean concentrations of As (3.6), Ba (377), Cr (16), Cu (13), Fe (8720), Mn (230), Ni (14), Pb (4.7) and Zn (75) mg/kg were all below the Canadian Council of Ministers of the Environment (CCME) interim freshwater sediment quality guideline (ISQG), where available. The concentrations of Cd, Hg, Sb and Se in all the samples were below the limits of detection. As, Cr, Pb and Zn in a few sediment samples exceeded the ISQG. There were no discernible statistically significant relationships between the surrounding land use and surface water quality parameters including the pH (which ranged from 7.66 to 8.98), and electrical conductivity (485 to 2940 $\mu\text{S}/\text{cm}$). Ongoing work include determining sediment physicochemical properties such as pH and total organic carbon content following which the relationship between sediment physicochemical properties and PTEs concentrations will be explored along with how the potential ecological and human health risk associated with sediment during recreational use. The relationship between PTEs concentrations and surrounding land use will also be explored using contamination indices (enrichment factor, contamination factor, geo-accumulation indices and pollution load index), along with source apportioning through correlation and principal component analysis. Finally, recommendations on limiting exposure to potentially contaminated sediments will be developed with the aim of achieving the United Nation's Sustainable Development Goals.

3.09.P-Tu235 Pollution Sources of PCBs, HCHs, and PFASs and Potential Risks of Soil Pollution

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Soil pollution represents a major environmental concern for maintaining soil health and soil ecosystem functioning. Among various pollutants, Persistent Organic Pollutants (POPs) are particularly problematic for soil health due to their persistence, bioaccumulation, and toxicity in soil. Various anthropogenic activities, including industrial operations, improper chemical storage and handling, and inadequate waste management, significantly contribute to soil pollution. Here we provide an overview of activities contributing to the pollution of soil by hexachlorocyclohexane (HCHs), polychlorinated biphenyls (PCBs), and per- and polyfluoroalkyl substances (PFASs), using different information sources. These sources include national databases of contaminated or remediated sites, national lists of potentially polluting activities, and relevant literature. Subsequently, EU-wide databases obtained from national and international inventories were used to map spatial

patterns and clustering of identified pollution causing activities within the EU.

The poster will present the clusters of these activities, co-occurrence of multiple pollution sources within the same geographic area at a high resolution of 1 km². The results effectively serve as a proof of concept to demonstrate the spatial co-occurrence of pollution-causing activities posing risks of co-contamination. The evidence provided by these datasets and the mapping approach used supports extensive and non-targeted soil monitoring, targeted decision-making for effective remediation, and environmental management practices.

3.09.P-Tu236 Combined laboratory and field evidence of neurotoxicity induced by metal(loid) mixtures and nanoparticles from metallurgical particulate matter

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Metallurgical industries emit settleable particulate matter that reaches aquatic systems and dissociates into metal and metalloid elements and metallic nanoparticles. These particles can accumulate in fish tissues and affect the cholinergic system. This study evaluated the accumulation of 27 elements and the presence of metallic nanoparticles in the brain and muscle of Nile tilapia after 96-hour exposure to environmentally relevant concentrations of particulate matter (0.0–1.0 g L⁻¹), and in fish collected from two contaminated lagoons and an aquaculture reference site in southeastern Brazil. Acetylcholinesterase activity was measured to assess neurotoxic responses.

In laboratory exposures, chromium, manganese, iron, nickel and barium increased in the brain in a concentration-dependent pattern, while aluminum, titanium, rubidium and tin increased in muscle. Transmission electron microscopy revealed iron, aluminum, copper, titanium and tin nanoparticles in both tissues of exposed fish and in field-collected animals from contaminated lagoons. In the laboratory, acetylcholinesterase activity increased in the brain at low and high particulate matter concentrations, whereas muscle activity decreased across all treatments.

Field-collected fish showed site- and year-dependent patterns. In the lagoon affected by atmospheric emissions, muscle acetylcholinesterase activity was consistently reduced, coinciding with the presence of tin and copper nanoparticles. In the lagoon receiving industrial effluents, brain acetylcholinesterase activity increased, suggesting excitatory neurotoxicity. Linear regressions revealed positive associations between brain acetylcholinesterase activity and zinc, zirconium, tin, barium and copper, and negative associations with silver and lead.

These results show that metal(loid) mixtures and metallic nanoparticles from metallurgical emissions bioaccumulate in edible fish tissues and alter enzymatic activity in both acute and chronic exposures. Such disruptions may impair behavior, swimming performance and survival, highlighting the need for regulatory control of metallurgical emissions to protect aquatic wildlife.

Disclaimer/Disclosure:

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3.10.A Experimental, Modelling, and Monitoring approaches to assess the Environmental Fate and Exposure of Pesticides

3.10.A.T-01 Update on improving the procedure for fitting degradation rates in water sediment studies

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In aquatic risk assessment studies it is typical to determine total system degradation and/or dissipation half lives from water-sediment experiments rather than individual degradation rates of the water and sediment phases. The

disadvantage of this is that the use of total system half lives or dissipation half lives may mischaracterise the environmental fate processes in aquatic systems, and may lead to unrealistic outcomes, especially if behaviours over long time frames are considered (e.g. FOCUS SW repair).

The reason that compartment-specific degradation rates are often not used in aquatic risk assessments is that it is difficult to obtain statistically robust quantification of these coupled rates in the experimental system (e.g. excluding M0, four rate parameters need to be fitted from approximately 8 - 12 time points), whereas it is easier to obtain total system degradation or dissipation half lives (e.g. excluding M0, one rate parameter is fit for in single first order kinetics).

We propose to use the sediment water sorption relation (expressed as K_d) to reduce the number of fitting parameters and thus increase the reliability of statistically robust fits. This method represents a simplification of the existing fitting method in that the fitting procedure has simply been reordered but allows for the decoupling of degradation and sorption and hence more realistic parameterisation of surface water models.

In previous work we have presented this approach applied to compounds where the sediment K_d was measured, and demonstrated that this approach is successful in obtaining robust fits. This resulted in robust compartmental half lives (level PII endpoints) compared to the use of conservative 1000 d defaults (arbitrary value stated in guidance) in conjunction with the total system half life (level PI endpoints).

In this present work, we now apply the approach to a range of regulatory compounds where the sediment K_d has been extrapolated from existing soil sorption data and consider a variety of optimisation weighting functions.

3.10.A.T-02 Effect of linear and Freundlich isotherms in the prediction with VFSSMOD of pesticide residue remobilization from vegetative filter strips

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Vegetative filter strips (VFS) are widely used at field edges to reduce surface runoff and associated eroded sediment and pesticide loads. Their mitigation effect has been incorporated into higher-tier EU FOCUS regulatory assessments through the SWAN tool, which couples the VFSSMOD model with the field runoff model PRZM and the surface water model TOXSWA. VFSSMOD is a mechanistic model that simulates runoff reduction by densely vegetated areas, accounting for VFS size, vegetation composition, hydrology, soil properties, and pesticide characteristics. The model, widely validated, estimates pesticide retention in dissolved and sediment-bound forms and considers partial remobilization of residues during subsequent runoff events, based on degradation and sorption dynamics between storms. This approach captures realistic interactions for chemicals with varying sorption and persistence.

Currently, VFSSMOD assumes instantaneous linear sorption. In this work, we incorporate the non-linear Freundlich isotherm into key VFS mitigation processes: soil leaching, event-based runoff equilibrium, inter-event soil water equilibrium, and residue remobilization. We compare predictions under linear and Freundlich assumptions across a wide range of pesticides and apply global sensitivity analysis to evaluate the importance of isotherm selection within the broader variability of VFS systems.

Results show that the maximum availability of porewater pesticide residues for remobilization in the next runoff event depends on sorption characteristics. For low K_{oc} , although the chemical is readily available in porewater, most has leached below the surface during the previous runoff event, resulting in minimal remobilization. For high K_{oc} , the chemical remains near the surface (limited leaching) but is largely unavailable in porewater, again leading to minimal remobilization. Intermediate K_{oc} values create a “sweet spot” for remobilization. However, this sweet spot shifts with isotherm type where in almost all situations the non-linear Freundlich isotherm will lead to higher sorption compared to the linear isotherm as environmental concentrations (soil runoff) typically are not be high enough to cross the intercept between both.

This study highlights the need for careful selection of pesticide sorption isotherms to realistically quantify VFS mitigation performance.

3.10.A.T-03 FOCUS Surface Water Repair Stage 2 Version Control Testing – First insights

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FOCUS Surface Water Repair is a major revision of the original framework, introducing multi-year exposure calculations to capture variability in weather and application timing. It updates scenarios and aligns with regulatory developments, aiming for more realistic exposure predictions for aquatic ecosystems. Extension from one year to 20 years supports robust characterisation of annual variation in exposure peaks and patterns. In FOCUS Version Control Stage 2, the new software suite is tested according to protocol with selected test compounds and target usage patterns. This presentation offers an overview of testing status and a summary of results for a range of theoretical chemicals and uses. Testing remains ongoing and it is noted that changes to software may be required before regulatory release.

Test compounds cover a broad range of fate properties: sorption, degradation (DT50 1–10,000 days), water solubility, vapour pressure, metabolites. The illustrative testing in this abstract considers a compound characterised by highly mobility ($K_{foc} = 100 \text{ L/kg}$), impersistence in soil and sediment (DegT50 = 10 d), and persistence in water (DegT50 = 1000 d).

Seven crops were selected: potatoes, maize, cereals, apples, vines, oilseed rape, leafy vegetables. Each GAP specifies application rates, strategies (groundspray, airblast, granular), and timings (dates, intervals, growth stages).

Simulations use current test versions after Stage 1 (July 2024), including a number of adjustments since the EFSA report issued in 2020.

The multi-year framework improves characterisation of variability in exposure and patterns. Values for risk assessment depend on a currently undefined protection goal percentile metric. Maximum PEC_{sw} under FOCUS SW Repair is typically higher than current scheme; the degree of divergence from the current framework depends on percentile and conditions simulated for representative year employed in the current scheme.

A key challenge for risk managers will be interpretation of variability across scenarios and crops. Stage 2 testing contributes to consideration of percentile thresholds and potential for customisation to scenarios. Analyses here are limited to one compound; broader results will follow and will be reported in the presentation.

3.10.A.T-04 GIS based modelling of linked aquatic exposure and effect modelling considering precision application

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Integrated landscape modelling of hydrology, chemical exposure and fate, alongside ecological effects, represents a higher-tier approach for refining surface water risk assessments of plant protection products (PPPs). Such modelling contextualises monitoring data and supports stewardship activities by incorporating local environmental and agronomic practices. Advances in digital farming and precision application techniques are increasingly relevant for regulatory risk assessment under EC Regulation 1107/2009. However, guidance on model and data selection is lacking, and further research is needed to demonstrate reliability and practicality in regulatory contexts.

This study presents a modular landscape modelling approach linking physically based exposure models with mechanistic effect models to evaluate the impact of precision application techniques, such as spot spraying, on aquatic ecotoxicological risk. The xLandscape framework was extended by integrating the Droplet and Atmospheric Dispersion drift (DAD-drift) model, which accounts for local conditions, nozzle characteristics, and agronomic practices. A statistical tool generated representative spot spray patterns based on infestation rates and patch size distribution. Surface water dynamics were incorporated to calculate realistic exposure profiles. A toxicokinetic-toxicodynamic model was adapted for Lemna sp., including habitat selection based on flow velocity.

The case study focused on early-stage herbicide application in oilseed rape within a meso-scale agricultural catchment (54 km²) in North Rhine-Westphalia, Germany. Simulations compared broadcast and spot applications (25% infestation) across 13 river segments. Standard FOCUS drift assumptions yielded PEC_{sw} up to 2.1 µg/L, whereas physically based modelling predicted concentrations below 0.1 µg/L. Spot application further reduced exposure by a factor of ~3.5. Risk assessment using EP50 values indicated failure under FOCUS assumptions but a high margin of safety for both broadcast and spot applications when using the refined approach.

This study demonstrates that linked exposure-effect modelling at the landscape scale significantly improves environmental realism and reduces uncertainty. Incorporating precision application techniques into regulatory assessments can support sustainable PPP use and local management strategies. Future work should address physical barriers and validation with monitoring data to enhance regulatory acceptance.

3.10.A.T-05 Harmonised Framework for the SETAC Spatially Distributed Leaching Modelling of Pesticides Initiative

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Spatially distributed leaching modelling (SDLM) of pesticides is a methodology to estimate the leaching potential over large spatial extents such as the national or European scale. While SDLM is already used for Tier 3B of the leaching risk assessment in some countries, a harmonised modelling framework for all EU member states does not yet exist. SDLM is also foreseen to be used in Tier 4 to help to select new groundwater monitoring sites in vulnerable regions and put existing groundwater monitoring sites into context. For these reasons, the SETAC-SDLM working group has recently developed a harmonised modelling framework. Two spatially distributed leaching models specifically developed for this project, based on the leaching models PEARL and PELMO, respectively. These models run up to 10,000 scenarios consisting of unique combinations of land-cover, climate, and soil data. The scenarios were derived from European soil and climate maps using k-means clustering.

At Tier 3B, these models are used to compute the 90th-percentile overall leaching concentration, which is the same exposure assessment goal as currently used in Tier 1 and 2 of the FOCUS groundwater report. The Tier 3B results have been aggregated on the FOCUS zone level and compared to Tier 1 results computed with FOCUSPEARL and FOCUSPELMO. Five example substances were tested and showed, which indicated that the leaching concentration at Tier 3B is generally lower than at Tier 1, but not for all FOCUS zones.

At Tier 4, groundwater monitoring studies must be carried out at locations that are sufficiently vulnerable in view of the existing FOCUS exposure assessment goal. The SDLM framework can play a crucial role for the selection of vulnerable regions in which to install monitoring wells and setting existing groundwater monitoring studies in context. Using site-specific soil, crop and climate data, the median annual mass flux to the groundwater can be estimated using regular leaching models. The median annual mass fluxes can then be plotted on the cumulative frequency distribution of the leached mass flux simulated with SDLM to assess whether the monitoring sites represent vulnerable locations.

3.10.B Experimental, Modelling, and Monitoring approaches to assess the Environmental Fate and Exposure of Pesticides

3.10.B.T-01 A Combined Deterministic–Stochastic Methodology for Estimating Residential Pesticide Exposure

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Residential exposure to agricultural pesticides remains a public health concern in intensively farmed regions such as the Netherlands. Airborne concentrations near treated fields arise from short-term spray drift and post-application volatilization, both of which contribute to downwind transport and potential exposure at nearby homes. Existing exposure models typically focus on single events and do not capture the full variability in meteorology, application timing, and field–house configurations that shape real-world exposure patterns. We developed a modelling framework that integrates first-hour spray drift (RTDrift), volatilization up to seven days after application (PEARL), and hourly Gaussian-plume dispersion to estimate outdoor residential concentrations. Exposure was evaluated for houses within 250 m of agricultural fields using BRP (fields) and BAG (residences). Downwind alignment was assigned using hourly wind direction ($\pm 45^\circ$). Eighteen houses from the 2017 OBO study were linked to one or more of seven nearby fields that met these criteria. Model performance was first evaluated using the documented 2017 OBO application events. Each house had one measured concentration per pesticide, and RMSE, MedAE, and bias were calculated per house and summarised as medians. A second evaluation used probabilistic exposure distributions generated by simulating

all legally and meteorologically valid field–house–hour applications in 2017 (16,135 scenarios). For each house, the measured concentration was compared with the predicted median concentration across all plausible application scenarios.

For acetamiprid, evaluation against the documented application events yielded a MedAE of 3.31 ng/m³, a median RMSE of 4.68 ng/m³, and a median bias of 3.30 ng/m³. For tebuconazole, MedAE and median RMSE were 5.18 and 7.04 ng/m³, respectively. In probabilistic evaluations, predicted median concentrations closely aligned with measured values, with median errors below 0.05 ng/m³ for both pesticides.

This study presents an exposure-modelling approach capable of capturing both event-specific behaviour and the broader distribution of realistic agricultural and meteorological conditions. The framework improves representativeness across real-world variability and supports future population health assessments of residential pesticide exposure.

3.10.B.T-02 Seasonal Dynamics of Pesticide Residues in Soils: Insights from Two-years of Monitoring in Three Agricultural Settings

Romualdus Kasteel, Vanessa Reiningier and Thomas Poiger, Agroscope, Switzerland

The European Soil Monitoring Law recently adopted by the European Commission emphasized the need to monitor emerging contaminants in soils, such as pesticides, which are recognized as a major cause of chemical pollution in agricultural soils. Whilst extensive monitoring campaigns have been conducted across Europe, these typically focus on single-time sampling at numerous sites. National monitoring programs would expand these campaigns to periodic repetition on annual or multi-year intervals. Intra-annual (seasonal) dynamics of pesticide residues in soils, however, remain largely unexplored. This study addresses this gap by performing monthly soil sampling over two years at three agricultural sites in Switzerland: an apple orchard, a vineyard and arable land cropped with potatoes and winter wheat. Samples were collected at depths of 0–5 and 5–20 cm. Residues of 38 active substances and 8 metabolites were extracted from soil samples by accelerated solvent extraction using an acidified organic solvent mixture and quantified by high-performance liquid chromatography-tandem mass spectrometry. Two cases were distinguished, with active substances being applied either during (12 compounds across sites) or (long) before the monitoring study. For the first case, dissipation from the 0-20 cm layer was modeled using a modified single first-order kinetic approach to account for observed accumulation at low residue concentrations. For the second case, multiple linear regression was employed to elucidate the contribution of seasonal variations in environmental and soil properties ? soil temperature, soil moisture, total carbon content, and the gravimetric moisture content after drying at 40°C ? on variations in residue concentrations. As opposed to the vineyard and arable land, an increase in summed residue concentrations was observed in the apple orchard, also during the winter period, and mainly attributable to the fungicides. Soil temperature was the dominant factor to explain variations in residue concentrations in the apple orchard, whereas it was total carbon content in the vineyard. No significant differences were observed for arable land, probably due to the homogenization by ploughing. To conclude, monthly soil sampling revealed that seasonal variations in residue concentrations of active substances that were applied long before the monitoring campaign can be linked to variations in environmental and soil properties.

3.10.B.T-03 Agricultural Practices Drive Pesticide Residues in Soil and Earthworms

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Farmland birds often have a sensitive conservation status and rely on agricultural landscapes where multiple pesticides are routinely applied. Despite the diversity of substances and large-scale application, direct links between pesticide usage and farmland bird trends remain poorly understood. In this study, current-used pesticide concentrations were quantified in soil derived from farmland bird habitats, and in a key dietary item: earthworms. By relating measured concentrations to site-specific agricultural practices and historic land-use, this study aims to provide insight into pesticide exposure in landscapes essential for farmland bird species. Samples were collected from 26 agricultural fields in the province Friesland, the Netherlands, in June-July 2020. The plots belonged to eight farmers and represented a gradient from extensive to intensive agricultural management. Historic land-use and management practices over the past 10 years were reconstructed through interviews with landowners. The plots consisted of four crop types: bulbs (n=4), corn (n=7), grassland (n=8) and old-grassland (n=7). All samples were screened for 648 pesticides. Fungicide concentrations were highest in bulbs, while herbicide concentrations were highest in corn. This likely reflects both crop type and timing of sampling as several corn fields were sprayed during sample collection whereas bulbs had already been harvested. Some grasslands showed fungicide levels exceeding those in corn, corresponding with past bulb cultivation and indicating long-term persistence of compounds. Bulb fields were associated with relatively high

levels of tebuconazole, hexaconazole, prothioconazole-desmethio, metazamitron and pendimethalin whereas corn was associated with dimethenamid, fluroxypyr-1-methylheptylester, foramsulfuron, fluroxypyr, and methiocarb-sulfoxide. A single pesticide was associated with old-grassland; bixafen. Insecticides were rarely detected. Although this study covered a single time point and didn't capture seasonal variability, the following conclusions can be drawn: i) crop type strongly structured pesticide profiles, ii) pesticide residues in grassland often reflect historic crop rotations, iii) management practices correlate with pesticide occurrence, and iv) quite similar patterns in soil and earthworms suggest that soil concentrations may provide a useful starting point for estimating potential pesticide exposure in farmland birds through dietary intake.

3.10.B.T-04 Determining Pesticide Concentrations in Water Framework Directive Water Bodies in the Netherlands

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Environmental quality standards under the Water Framework Directive (WFD) aim to protect the chemical and ecological status of European surface waters. Several active substances in plant protection products (PPPs) fall under these standards, which are often more stringent and based on different protection goals than those used in authorization procedures. As a result, PPP applications that comply with legal use conditions may still lead to exceedances of WFD standards in receiving water bodies. This work addresses the need for a method that links edge-of-field authorization assessments to expected concentrations in Dutch WFD water bodies.

We develop a rapid assessment tool that predicts how agricultural loads move from treated fields to edge-of-field watercourses, through sub-catchments, and ultimately to WFD water bodies. The approach has two components: First, it estimates local loadings entering watercourses via spray drift, runoff, erosion and drainage. Spray-drift inputs are calculated using the WUR Drift Calculator combined with high-resolution crop and watercourse maps. Runoff-related pathways follow the structure of FOCUS Surface Water Steps 1 and 2, where soil residue dynamics, crop interception, precipitation timing and degradation determine the available mass.

Loadings are allocated to watercourse segments based on treated crop area, and weekly mass inputs are generated for each sub-catchment. Second, these loadings are routed downstream using the WFD Explorer, which accounts for hydrological connectivity, transport, dilution and dissipation. A transfer-matrix approach is then used to derive maximum and average predicted concentrations for each receiving WFD water body, allowing comparison with relevant WFD environmental quality standards.

The method provides a transparent framework that links PPP use at the field level to concentrations at the scale of WFD water bodies. It enables identification of crop–substance–catchment combinations likely to exceed standards and highlights the key drivers behind high concentrations. Challenges encountered include handling substances with high sorption, managing detailed spatial datasets and aligning crop classifications across regulatory systems. Simulations for example substances illustrate how hydrology, crop patterns and substance properties influence concentration outcomes. The tool is designed to provide a basis for early regulatory risk assessment.

3.10.B.T-05 From Source to Tap: Uncovering the Origins of 1,2,4-Triazole in Flemish Drinking Water

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Elevated concentrations of 1,2,4-triazole, a metabolite of a variety of substances, were detected in drinking water production centres in West-Flanders, Belgium. These facilities rely on surface water, making them vulnerable to contamination, especially in large, cross-boundary catchment areas. Identifying the origins and different transportation routes of 1,2,4-triazole to the surface water is essential to make informed decisions on mitigation strategies and to be able to assess their efficacy.

To address this challenge, a multi-method approach was applied, combining literature review, spatial modelling, targeted monitoring, and advanced analytical techniques. Potential sources and parent compounds were identified through a literature review, and pathways to surface water were mapped. A theoretical pressure map for diffuse contributions from plant protection products was developed using spatial modelling based on FOCUS Step 1 principles, incorporating crop distribution, permitted pesticide use, and chemical properties. Time-series analysis of monitoring data was combined with Non-Target Analysis (NTA).

Results revealed clear spatial and temporal patterns in 1,2,4-triazole concentrations. Point-source monitoring

identified contributions from food-processing industries and wastewater treatment plants, resulting in the highest concentrations in both surface water and treated drinking water. In areas without point sources, where agriculture was the sole contributor, minor exceedances of the drinking water limit were observed. Agriculture dominates land use in all abstraction zones, with highest diffuse pressure from plant protection products in the upstream areas linked to cereals, potatoes, and vegetables. A marked increase in 1,2,4-triazole concentrations was observed during drinking water production, with the largest increase during summer. These increases could not be explained by targeted analysis of known parent compounds. NTA revealed intermediary compounds that degrade into 1,2,4-triazole during treatment, highlighting complex transformation pathways.

This study demonstrates the value of integrating spatial modelling, targeted monitoring, and advanced analytics for source identification of newly identified micropollutants in drinking water. Furthermore, this methodology is transferable to other contaminants of emerging concern, bridging scientific research and mitigation strategies for proactive risk management.

3.10.P Experimental, Modelling, and Monitoring approaches to assess the Environmental Fate and Exposure of Pesticides

3.10.P-Tu238 Comparison of Different Approaches to Simulate Runoff and Erosion for Use in Spatially Explicit Models

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For the authorization of plant protection products, the environmental exposure assessment for runoff and erosion is conducted based on the mostly empirical model PRZM. Herein, pre-defined scenarios for location, weather, soil management and crop are used. While this being a well-established procedure, further development towards more spatial explicit modelling has gained traction in recent years. PRZM uses the runoff curve number (RCN), the models ZIN-AGRITRA, openLISEM and RZWQM2 use mechanistic approaches. That raises the question of whether and to what extent the use of more mechanistic modelling approaches in conjunction with field-specific assessments leads to a more refined exposure assessment. In the present study, we compared the capability and performance of the four models to simulate observed edge-of-field runoff and erosion. We investigated the hypothesis that an increase in model complexity implemented in the more mechanistic and comprehensive models yields to more realistic and accurate simulations. First results prove the assumption of higher accuracy due to higher resolved data and more mechanistic process implementation. However, this comes at the expense of the need for additional data that is either not available, or the model still needs to be calibrated to realize the gain in precision compared to simpler empirical models. Therefore, the analysis requires also other criteria such as scalability, technical feasibility, availability of data, desirability, and economic viability to come to a robust conclusion of the gains and limitations of field-specific modelling approaches. But this will only be evaluated in the later stages of the project. To reproduce field observations, all models often require case-by-case parameter calibration, rendering predictive assessments challenging. However, in future exposure assessments, the use of more comprehensive models is deemed to be useful as a refinement to standard calculations and for specific fields under investigation. Nevertheless, the PRZM RCN approach with the lowest number of parameters represents a reasonable trade-off between accuracy and input data demands for applications on a large scale without the possibility to acquire or calibrate additional parameters that are required by the other models.

3.10.P-Tu239 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 DegT50_{field, equilibrium} (rescaling of DegT50_{field}) for input in exposure models in combination with from laboratory aged sorption studies derived parameters (fNE, kdes) as described for DegT50_{laboratory} values. This virtually prevents the mitigating effect of aged sorption on leaching and limits the applicability of the aged sorption guidance in combination with DegT50_{field} 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. The detailed evaluations of the datasets illustrate the relevance of aged-sorption under field conditions.

Based on the evaluation of the field datasets, revisions are proposed to the current EU Aged Sorption Guidance fully allowing for the combination of field degradation and aged-sorption data including a modified flowchart (Figure 5-4 in the Guidance Document).

The revisions are: If field and laboratory DegT50 datasets are from different populations according to the EFSA DegT50 endpoint selector, then checks are made [P5-metric or Kd_app] to test if there is evidence of aged-sorption under field conditions. If there is evidence, then the geometric mean normalised DegT50_{field, equilibrium} is used in combination with the mean laboratory aged sorption parameters, FNE and k_{des}, 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 combined in exposure assessments and Tier-1 approaches should be retained.

A report on the additional field work has been issued and the proposed revision of the EU Aged Sorption Guidance Document sent to the EU Commission and EFSA for evaluation and acceptance. The changes proposed to the GD will be discussed.

3.10.P-Tu240 Experiences with the EFSA/ECHA Water Treatment Guidance

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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 seven 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 6) sand filtration and 7) biodegradation. These tests are to be conducted at environmental related concentrations in the range of 1-10² 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.

This presentation shares the experience of CropLife Europe Member Companies in the implementation of the Water Treatment Guidance, including harmonisation of experimental testing protocols for the conduction of treatments and analytics according to the new guidance, through the use of cases studies and best practice derived from ongoing experimental studies.

Disclaimer/Disclosure:

The authors declare no competing financial interest.

POH, DP, TB, MS, SW and MK are employed by a chemical and/or PPP manufacturing company.

3.10.P-Tu241 Catchment-based Pesticide Vulnerability Mapping: Contextualizing Surface Water Monitoring Using two Methods in Flanders (BE)

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Similar to other regions with intensive agricultural activity, frequent exceedances of pesticide quality standards in Flemish surface waters highlight the need for spatially explicit exposure assessment tools that go beyond

local monitoring. The susceptibility to pesticide contamination, referred to as catchment vulnerability, reflects the likelihood of pesticide transport to surface water bodies and varies significantly between catchments depending on environmental characteristics such as soil properties, topography, hydrology, and agricultural practices. While established methods exist for groundwater vulnerability assessment, comparable approaches for surface water are lacking.

This study introduces and compares two complementary methodologies for ranking catchment vulnerability to pesticide contamination in surface water: a newly developed hybrid GIS-based model and the process-based SWAT+ model. Vulnerability maps were generated for three herbicides (flufenacet, ethofumesate, metribuzin) across Flanders (Belgium) and validated against monitoring data from 237 catchments (2017–2021) and detailed time series from two intensively monitored sites.

Correlations with observed concentrations and loads were moderate to strong for flufenacet and ethofumesate, while metribuzin exhibited greater variability due to localized crop use and summer application timing. SWAT+ captured seasonal dynamics and event-driven peaks without site-specific calibration. The highest vulnerability to pesticide losses was observed in the southwestern and hillier southern parts of Flanders, driven by a combination of topography, land use, and hydrological factors. Despite using two methodologically distinct approaches, vulnerability patterns agreed well, emphasizing the robustness of the approaches.

This work addresses an important gap in surface water vulnerability assessment, where standardized comparative frameworks remain limited compared to groundwater assessment methods. By enabling a relative ranking of catchments, these methods support regional-scale risk management, facilitate selection of representative monitoring locations for regulatory purposes and allow optimized implementation of mitigation strategies to protect water quality. The methods are transferable to other regions and contribute to the growing need toward more spatially explicit and systems-based approaches in pesticide regulation.

Disclaimer/Disclosure:

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3.10.P-Tu242 A Proposal for Harmonized Gathering of EU Surface Water Monitoring Data of Plant Protection Products

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Surface water (SW) monitoring data play a crucial role in evaluating the ecological status of aquatic environments and identifying potential risks from chemical contaminants. In the case of plant protection products (PPPs), monitoring data are a component of renewal applications and are relied upon in emission reduction plans.

At present, there is no uniform strategy to monitor compliance of PPPs with SW environmental quality standards (EQS), i.e., no agreed essential requirements are identified for generating SW monitoring data that reliably serve this purpose, ensure data comparability across the EU, and support the development of mitigation measures to reduce surface water exposure to PPPs. This is currently reflected in the highly heterogeneous quality and quantity of SW monitoring data across the EU.

To support a reliable use of SW monitoring data of PPPs for contextualization purposes and evaluation against EQS, this poster aims to suggest a baseline for quality criteria.

This work highlights deficiencies in publicly available SW monitoring data across the EU, particularly in the context of assessing compliance of PPPs with EQS, and indicates that besides general information such as climate data, type of SW body, and flow velocities, substance-specific properties and further metadata are required to allow for a robust interpretation of the SW monitoring data and their reliable use in contextualization efforts. Proposed essential metadata include geographic coordinates, application profiles, soil types and topographical features, coverage of arable land and its spatial distribution, drainage infrastructure, potential industrial and urban emitters, and hydraulic conduits that may directly connect runoff from agricultural fields to adjacent SW bodies.

The protection of SW should be a shared interest of applicants of PPPs, competent authorities, land managers, and academia, and so should the development of a harmonized approach for generating reliable SW monitoring data to ensure data comparability and a robust assessment of compliance with EQS. Establishing harmonized monitoring practices would also support the contextualization of SW monitoring results, which, in turn, would

facilitate the development of effective mitigation measures to prevent PPP from entering into waterbodies. A coordinated SW monitoring strategy across EU Member States would significantly contribute to both regulatory consistency and environmental protection.

3.10.P-Tu243 IDENTIFICATION OF PESTICIDES ALONG THE FLYWAY: THE FIRST STEP IN A RISK ASSESSMENT FOR THE BLACK TAILED GODWIT

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Loss of habitat is pointed out as a key driver of bird population declines worldwide, however exposure to contaminants has been shown to potentially lead to immune and reproductive alterations that can have severe long-term populational effects. However, comprehending the risks of contaminants to wildlife species is often hindered by their exposure to multiple chemicals simultaneously and, for migratory animals, by the spatial/temporal variability of exposure. Therefore, the main goal of this study is to conduct a risk assessment for pesticide exposure in black tailed godwits (*Limosa limosa*), as a proof of concept for how risk assessment can be performed for migratory species in general. The first step toward that goal is to establish to what individual compounds and mixtures godwits are exposed at different locations during migration. To accomplish that, 81 soil samples were collected in different regions of Spain (Extremadura and Doñana), France (Camargue, Vendee and Charente Maritime) and Portugal (Tejo and Sado estuaries). These regions are frequently used as stop-over sites for black-tailed godwits during their annual migration, as evidenced by previous telemetry data. Samples were freeze-dried, homogenised and underwent pesticide extraction following the QuEChERS method with slight modifications. Extracts were analysed on a LC-MS/MS using a Shim-pack Velox Biphenyl Column (Shimadzu, Japan). The used method screens for 640 current use pesticides. Individual compounds were identified through comparison of retention time and mass-to-charge ratio (m/z) of detected compounds to those of standard analytes included in the analysis. 34 different compounds were identified, encompassing fungicides, herbicides and insecticides. Only 4 of them were detected in at least one sample of each country (3-Indolyl-acetic acid, tebuconazole, DEET and azoxystrobin), while 20 compounds were detected exclusively in one of the three countries. These preliminary results suggest that pesticide use varies per region, which is in accordance with the different crops being cultivated in each location. Nonetheless, this indicates that godwit exposure to pesticide is likely dependent on migration route and reinforces the need for taking spatial variability into account when assessing the risk of chemicals to migratory animals. Next steps in this study include quantifying the pesticides detected and expanding the sampling to include also wintering and breeding grounds of the species.

3.10.P-Tu244 Fate of Nitrification Inhibitors DMPP and Nitrapyrin in Agricultural Soils – Combination of Lab and Field Study

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Nitrification inhibitors (NIs) are recommended as a solution to reduce nitrous oxide (N₂O) emissions from agricultural fields to achieve the EU target to reduce greenhouse gas emissions by at least 55% before 2030. However, their environmental fate is only rarely studied and the risk of introducing a vast amount of additional synthetic organic chemicals into agricultural soil calls for a careful environmental assessment. However, the requirements for such assessments are relatively small, as NIs are not treated as plant protection products or biocides, but falling under the fertilizer regulation in the EU. Therefore, we evaluated the environmental fate of two NIs, DMPP and Nitrapyrin, the two compounds with the highest marked share in Denmark. In a combined lab and field experiment we studied the degradation kinetics and half live in different soil depths, and evaluated the effects of the addition of manure and formulating agents. Degradation of the NIs was observed as well as formation of their transformation products. While no effect was observed from the formulating agent for DMPP, the microencapsulation of Nitrapyrin slowed the degradation. Degradation in deeper soil layers was only slightly reduced compared to top soil. Nonetheless, although fast degradation was seen in the presence of manure both in lab and field studies, observed residual concentrations of 5-15% of the active ingredient 6 Months after application, pose the risk of accumulation of NIs in the topsoils of agricultural fields.

3.10.P-Tu245 Sorption and transformation of metazachlor and its metabolites in soils and their potential to contaminate groundwater

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Metazachlor is a widely used herbicide that is transformed into various metabolites that are often found in groundwater. To assess their mobility in the soil environment, batch sorption experiments were performed for the parent compound and the two most frequently occurring metabolites metazachlor OA (479M04) and metazachlor ESA (479M08) and eight representative soils of the Czech Republic. The experimental sorption isotherms were fitted with Freundlich equations. The resulting Freundlich sorption coefficients (KF) were related to soil properties. The dissipation of these three compounds in five soils was further studied. During standard batch experiments, the concentrations of all three compounds and two additional metabolites (479M09 and 479M11) were always measured. The first-order kinetic model was assumed to describe dissipation profiles of the applied compounds. In addition, the rates of metabolites' formation, i.e. their fraction from the original substance, as well as their subsequent dissipation of all four metabolites were calculated.

The KF coefficient of the parent compound was an order of magnitude higher than that for the metabolites OA and ESA. Thus, while metazachlor showed high and even very high mobility in soils, both metabolites showed an extremely high mobility in the soil environment. Sorption of all compounds was mostly affected by the soil organic carbon content. The sum of fractions of all four metabolites formed from the parent compound ranged between 70 and 95%. The rate of dissipation of the metabolites OA and ESA was an order of magnitude lower than that for the parent compound. The dissipation rates of other two metabolites were generally higher than those of the metabolites OA and ESA but considerably lower than those of the parent compound. Dissipation rates were soil type dependent. The highest rates were observed in Stagnic Chernozem and the lowest in Eutric Cambisol.

Resulting sorption and dissipation characteristics and the map of agricultural soils of the Czech Republic and related database were next used to derive maps of mobility index and stability index. Finally, specific groundwater vulnerability maps for each compound were obtained by combining the DRASTIC vulnerability index and both soil indexes. The maps were confronted with the occurrence of these compounds in the groundwater of the Czech Republic.

Disclaimer/Disclosure:

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3.10.P-Tu246 Influence of Groundwater Flow Rate on Organic Micropollutant Biodegradation in Drinking Water Aquifers

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Water quality in drinking water aquifers is threatened by the increasing presence of organic micropollutants (OMPs) such as pharmaceuticals, pesticides and household chemicals. Drinking water aquifers are biologically and geochemically diverse systems, where water extraction creates variations in flow rates and patterns towards extraction wells. Flow rate influences the contact time between OMPs and indigenous microbial communities mostly present as biofilm, as well as the flux of available dissolved organic carbon (DOC), nutrients and electron acceptors. Additionally, groundwater composition becomes less favorable to support microbial activity with depth, as DOC fractions and electron acceptors are preferentially consumed. In aquifers, the biodegradation of OMPs is either diffusion limited (insufficient substrate flux) or reaction limited (insufficient contact time). Little is known about the windows of opportunity in groundwater flow rate for OMP biodegradation in microbial communities across drinking water aquifers. This project aims to better understand the effect of groundwater flow rate in aquifers on OMP biodegradation. A 2D flow chamber with a glass front plate and steel frame (dimensions 130x80x15 cm) is fully saturated with wet sand and inoculated with groundwater. The reactor is run aerobically with a horizontal flow at a flow rate of 20 L/day (residence time 4 days) with 4 in- and outlets on either side. The system is operated in several different phases of 21 days where the removal of more than 20 OMPs at 1 µg/L is monitored through changes in flow rate, DOC dosing and/or oxygen spiking. Samples for OMP, DOC and microbial community analysis are taken from 16 sampling ports distributed on the glass plate and redox probes are used to monitor redox conditions. Laboratory results will be accompanied by reactive transport modelling conducted in COMSOL to give insight into reactor flow patterns and flow rates. We hypothesize that biodegradation of OMPs is influenced by variations in contact times and substrate supply,

but that the availability of oxygen is the biggest determining factor. Our findings will give insight into the fundamental physical and chemical mechanisms underlying OMP biodegradation in drinking water aquifers.

3.10.P-Tu247 Exploring the Leaching Dynamics of Aged Residues of Atrazine in Soils: Effects of Temperature and Flow Rate

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Although atrazine has not been applied to Swiss railway tracks since 1993, the herbicide and its metabolites remain detectable in many of these soils and continue to leach through these typically well-drained, low-sorption substrates. Here, we investigate how temperature and precipitation influence this leaching behaviour.

To assess atrazine and metabolite leaching under controlled conditions, we operated soil columns (ca. 20 cm² × 30 cm) packed with railway-track soil from two Swiss sites containing aged atrazine residues and its metabolites. A diluted CaSO₄ solution was pumped at varying flow rate. Each column was kept at a specific temperature, which could be changed during the experiment. Leachate was collected and analysed by LC-MS/MS. Additionally, to compare breakthrough curves of freshly added atrazine with that of aged atrazine, each column received a pulse of isotopically labelled atrazine (atrazine-¹³C₃-¹⁵N₃). Before the leaching experiments, sequential extractions with diluted CaCl₂, acetonitrile, and Soxhlet-acetonitrile were performed to characterize easily and poorly accessible residues. At the end of the experiments, acetonitrile-extractable residues were quantified again with Soxhlet in six column segments to determine the residual mass distribution and establish a mass balance.

Concentrations of atrazine and metabolites in leachate rapidly declined to <1 µg/L. Raising the column temperature led to increasing concentrations, whereas lowering had the opposite effect. A fivefold increase in flow rate (from 37 to 183 mm/day) led to lower leachate concentrations in both soils, indicating that the percolation rate exceeded the rate of desorption.

The spiked atrazine-¹³C₃-¹⁵N₃ responded similarly to temperature and flow rate changes as aged atrazine. Despite equal spike amounts, peak concentrations differed by an order of magnitude between the soils (ca. 0.7 vs. <0.07 µg/L), likely due to their different organic-carbon contents (0.37% vs. 0.79%). After approximately two months of column operation, the columns had received the equivalent of ca. 42 mm/day of rainfall, totalling ca. 2.5 m of precipitation. The organic extractions revealed that the soils still retained several times the mass of atrazine and metabolites that had leached.

The ability to characterize the leaching behaviour of long-term reservoirs of organic pollutants, such as atrazine, is essential for improving predictions of their mobility in railway-track soils.

3.10.P-Tu248 Environmental Fate of a Starch Nanocrystal Formulation for a *Bacillus thuringiensis* Biopesticide

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Conventional chemical pesticides are widely used in modern agriculture. However, due to excessive use, adverse effects have been observed in both human health and the environment. Therefore, biopesticides are considered highly promising alternative, but their large-scale use is currently hampered by a lack of suitable formulations. Moreover, these formulations may contain constituents like synthetic and nanosized particles, which in turn pose an additional threat to the environment. The knowledge of the fate of these nanosized particles in an agricultural context is currently limited. It is important to consider the formulations since the environmental behaviour depends not only on the active ingredient but also on its formulation. One approach to minimizing environmental impact is to utilize bio-based formulations.

Therefore, we aim to investigate the fate of a starch nanocrystal (SNC)-based formulation for a *Bacillus thuringiensis* biopesticide using a soil column experiment in outdoor conditions. We will use the Pickering emulsion technique to encapsulate the active ingredient, with the SNCs acting as a stabilizing agent. The fate is analyzed using the radioactive carbon isotope (¹⁴C) by labelling the SNCs, which will allow us to track their transformation, distribution, and degradation. Through the application balance and the residues of the

formulation components in the different layers of soil, leachate, and plants, we will make precise statements whether the ingredients are integrated into the matter cycle of the soil, whether they are washed out, or whether they are taken up by the plants. Additionally, the impacts of formulation will be assessed on the soil enzyme activity and microbial community. An entire growing season of spring wheat (*Triticum aestivum*), including two test soils and biopesticide concentrations, ensures a wide range of environmental variability.

We hypothesize that the initial degradation of the formulation will be rapid; however, the SNCs may be taken up by the plants due to their size. As the environmental fate of bio-based nanoformulations in agricultural soil-plant systems has not been assessed before, this study will provide valuable insights into the interactions between the material and soil. The findings will provide support for regulations for biopesticides and formulations, ultimately ensuring their safe and sustainable use.

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3.10.P-Tu249 Modelling Airborne Pesticide Spray Drift for Orchard Sprayers

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Spray drift occurs when pesticide particles are carried away by wind from their intended target location to a neighboring area during or immediately after application. The issue of off-target spray drift with pesticides applications is a concern both to public and environmental health regarding the potential adverse health effects on humans (e.g. residents), as well as hazardous environmental impact to wildlife and water sources. Accurate drift measurement and prediction thus play a key role in risk assessment of spray applications. However, quantification of airborne spray drift is challenging because of its high variability related to environmental conditions, spray application technology, canopy structure, and measurement procedures. Over the last 30 years, a number of different approaches have been taken, mainly to modelling spray drift deposition onto the ground (i.e., dispersion models, computational fluid dynamics models, droplet tracking models and regression models), where each approach has its pros and cons. The aim of this study is to develop a model to quantify and predict non-target airborne exposures to pesticides due to spray drift from conventional cross-flow sprayers for fruit orchards. Generalized additive models (GAMs) are one of the most widely used and powerful modelling techniques in environmental studies for revealing non-linear effects of the explanatory variables on the response variable. In this study, GAMs are used to examine the relationship between downwind airborne drift concentration at different heights and meteorological conditions (i.e., wind speed and direction, relative humidity, air temperature), at various canopy growth stages of the orchard trees, and corresponding sprayer settings, by fitting the model to field trial data gathered over 20 years in the Netherlands. Model choice is via the Akaike Information Criterion (AIC) and the final model is validated in a cross-validation approach. The statistical characteristics of airborne drift concentration from the trials will be described. Next, the construction of the final model (i.e., the explanatory variables from the selection process) will be presented. The contribution of meteorological quantities and canopy development stage to the model, its validation results and goodness of the fit will be presented and discussed in detail. Main conclusions will be summarized.

3.10.P-Tu250 Enhancing Spray Drift Assessment: Integration of Vertical Drift Distribution in the Casanova Drift Model for Non-Target Organisms Risk Evaluation

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Spray drift modeling has become increasingly important in the regulatory assessment of crop protection product applications, particularly as concerns grow over the potential impacts on non-target organisms, including non-target arthropods (NTAs) and non-target terrestrial plants (NTTPs), as they play critical roles in maintaining biodiversity, ecosystem services, and agricultural productivity. Regulatory frameworks demand accurate predictions of drift behavior to ensure the safety of ecosystems adjacent to agricultural fields. Assessing the risks to NTAs and NTTPs remains challenging due to the limitations of current models, which primarily

generate spray drift deposition curves without accounting for vertical distribution of spray drift e.g. in plant canopies off-crop. This study addresses these challenges by leveraging the mechanistic Casanova spray drift model, which provides enhanced simulation capabilities to estimate not only deposition curves but also vertical drift profiles. By solving coupled ordinary differential equations (ODE) that account for drag forces, evaporation, and wind profile interactions, the model captures the complete three-dimensional transport of droplets from nozzle release through deposition. This enables extraction of vertical drift concentration profiles at specified downwind distances, providing critical exposure data for organisms residing at different canopy heights. Preliminary results indicate that the Casanova model can effectively produce short-range aerial drift patterns which could be used to predict off-crop capture by non-target species. In conclusion, the Casanova drift model represents a valuable tool for addressing the complex challenges associated with assessing spray drift impacts on NTAs and NTPs. By generating both deposition curves and vertical drift profiles, this model will aid in the development of effective strategies to protect non-target organisms in the context of crop protection applications.

Keywords: Spray drift modeling, vertical drift distribution, Casanova drift model, non-target arthropods, non-target terrestrial plants, regulatory risk assessment, environmental risk assessment

Disclaimer/Disclosure:

The Casanova drift model was originally developed by Bayer AG. The extraction of vertical drift concentration profiles from the model was financed by CropLife Europe aisbl.

3.10.P-Tu251 From 2D to 3D: using models to increase realism in exposure assessment of NTTO

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EFSA has been mandated by the European Commission to revise the environmental risk assessment (ERA) for non-target terrestrial organisms (NTTO), with a particular focus on minimizing uncertainty in pesticide exposure estimates. Current regulatory approaches rely on flat surface deposition assumptions, which limit the characterization of exposure under realistic agricultural conditions (i.e. three-dimensional exposure within and beyond crop canopies). For a holistic ERA, vegetation exposure must be conceptualized as a 3D process encompassing in-field overspray with vertical dispersion and off-field spray drift involving horizontal deposition and vertical dispersion.

To tackle these gaps, the CLE NTTO Drift Ad Hoc Group launched a collaborative project with two main pillars: (1) Develop fit-for-purpose models describing droplet dispersion and spray drift at different tiers, and (2) Generate experimental data for model validation. Modeling efforts include adapting existing tools and developing new approaches, such as extending current drift deposition models for canopy-level prediction (e.g. extending Casanova) and exploring computational fluid dynamics (CFD)-based solutions for within-canopy droplet behavior. Simplified meta-models are also being designed to enable broader use. In parallel, wind tunnel experiments aim to generate quantitative data for validating these models.

This exercise faces several challenges: the complexity of simulating droplet behavior within heterogeneous canopies, the scarcity of data for model calibration, and the need to balance scientific rigor with practical usability. These challenges highlight why collaborative efforts and transparent dialogue are essential to progress.

This poster will present the rationale for this initiative, summarize the regulatory drivers for action, and describe the envisioned pathway toward harmonized, science-based NTTO exposure assessments.

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3.10.P-Tu252 Land Surface and Pesticide Transport Modelling

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Soil pollution is an environmental concern in Europe. The European Commission (2020) reported that around 60–70% of European soils are unhealthy, highlighting the need to understand pollutant sources, transport processes, and mitigation strategies. The EU-funded SOILPROM project addresses this need by delivering integrated models for pollutant transport to assess the impacts of soil pollution on soil functions and ecosystem services. Our study simulates the spatial distribution and long-term evolution of pesticide concentrations in groundwater based on surface applications. Pesticide transport in the vadose zone is generally simulated using 1D models, such as HYDRUS-1D. A key challenge in vadose-zone flow and transport simulations is the accurate representation of bottom boundary conditions in the models. These boundaries are typically formulated as pressure heads corresponding to the water-table depth, which is largely unknown across Europe. To address this limitation, we use the hydrologic model ParFlow, which simulates 3D variably saturated groundwater-surface water flow in the coupled groundwater-soil-vegetation system for a domain covering Germany and neighboring regions. In the model, water table depth is a diagnostic variable that varies freely in response to 3D transient watershed hydrodynamics. We evaluate an approach that couples HYDRUS-1D simulations with ParFlow-derived water-table fluctuations. HYDRUS-1D was setup for 174 European Climate Assessment & Dataset (ECA&D) sites over Germany. We also compared the impact of selecting the atmospheric top boundary conditions, i.e., precipitation and reference evapotranspiration, in HYDRUS-1D, based on ECA&D observations or ERA5-Land reanalysis. A comparison between ERA5-Land and ECA&D evaluates the suitability of ERA5-Land for future recharge and pollution risk simulations. Results show that ERA5-Land captures temporal climate dynamics well, but smooths spatial variability. Initial HYDRUS-1D results show high spatial variability in bottom-water fluxes driven by climate, soil properties, and water-table dynamics, all of which strongly affect pesticide leaching. We will then extend the work to model pesticide transport in soil and groundwater. HYDRUS-1D will generate bottom-flux pesticide concentrations and mass fluxes, which ParFlow will use to model coupled flow and transport in groundwater, enabling a full assessment of pesticide fate in the subsurface water continuum from the surface to groundwater.

3.10.P-Tu253 Advancing Precision Application Modelling: Development of a Patch Generator for Realistic Infection Pattern Simulation

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Precision application of plant protection products is a key strategy for reducing environmental exposure while maintaining crop protection efficacy. Capturing the fine-scale spatial variability of infection and application patterns is essential for modelling precision pesticide application and associated environmental exposure. Conventional approaches often assume random or homogeneous infection distributions, which do not fully represent the spatial complexity observed in real agricultural systems. To support such analyses, we developed a Patch Generator capable of producing synthetic but realistic infection maps that capture the dual-scale (fine and broad) spatial organization of infections typically observed in agricultural fields.

The generator is defined by a set of intuitive parameters controlling infection rate, edge bias, and spatial clustering at fine and broad scales. It combines stochastic noise generation with spatial smoothing to create continuous infection patterns from discrete inputs. A micro–macro ratio parameter controls the relative contribution of small and large infection clusters, allowing the creation of maps ranging from scattered micro-spots to larger aggregated patches. This flexibility enables the generation of coherent, edge-influenced infection patterns that closely resemble those observed in field measurements.

High-resolution infection and application maps (0.25×0.25 m) were used to guide the generator's development. The observed patterns were analyzed to derive representative parameter inputs, enabling a direct link between field data and simulated maps. Visual and spatial comparisons showed that the generated maps reproduce realistic clustering and edge effects, providing robust inputs for simulation studies in fields lacking direct observations.

The Patch Generator offers a versatile and transparent method for representing infection variability within agricultural fields. By generating realistic synthetic maps from a limited set of parameters, it enables the implementation and assessment of precision application scenarios within existing modelling frameworks, enhancing the realism of spatially exposure simulations.

3.10.P-Tu254 Predicting Photodegradation of Pesticides in Swedish Waters: Validation of a Mechanistic Model

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Photodegradation can be an important transformation process affecting the fate of pesticides in environmental waters, and therefore quantifying photodegradation is needed to support environmental risk assessments. However, rates of direct photolysis and indirect photodegradation – driven by photochemically produced reactive intermediates (PPRI) formed when chromophoric dissolved organic matter (CDOM) absorbs sunlight – vary depending on environmental conditions such as sunlight intensity and CDOM composition. To account for this variability in environmental conditions, mechanistic models can predict photodegradation rates across diverse waterbodies. Yet, the ability of these models to reliably predict photodegradation rates in real environmental waters remains uncertain. In this study, water samples were collected from 14 sites across the Stockholm region (including lakes, streams, and coastal waterbodies; dissolved organic carbon (DOC) = 6.2–30 mg/L) to validate a mechanistic photodegradation model. This model predicted indirect photodegradation rate constants for over 20 pesticides based on measured steady-state concentration of three key PPRI: $^1O_2$, $^{\bullet}OH$, and $^3CDOM^*$ and previously determined bimolecular reaction rate constants for each pesticide-PPRI pair. Direct photolysis predicted rate constants were calculated using previously measured quantum yields and molar absorptivity values. The combined predicted photodegradation rates were then compared with observed rates measured in all water samples. Results showed that model predictions for pesticides primarily degraded by $^{\bullet}OH$ were generally within a factor of 3 of the observed values. While the model suggested that many pesticides could also react with $^3CDOM^*$, consideration of the photochemical properties of $^3CDOM^*$ was needed to determine whether these reactions occurred in the water samples and which sensitizer should be used in the model as $^3CDOM^*$ proxy. Overall, this mechanistic photodegradation model allows estimating site-specific photodegradation rates under varying environmental conditions, providing a tool to advance our understanding of pesticide persistence in environmental waters. Integrating such approaches into regulatory frameworks could strengthen environmental risks assessments and support informed pesticide management strategies.

3.10.P-Tu255 A Higher-Tier Approach: Kinetic Evaluation of Laboratory Water/ Sediment Studies at P-II Level

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P-I level (i.e. total system) endpoints are usually applied for predicting environmental concentrations of active substances in surface water and sediment in the context of the EU risk assessment of plant protection products (PPP). Standard P-II level kinetics are often limited in their applicability as refinement option due to unrealistic consideration of the exchange processes between the compartments. However, current regulatory developments (e.g. FOCUS surface water repair action and EFSA drinking water treatment assessment) require reliable P-II level endpoints to avoid over-conservative risk assessments.

In this study, we present a higher-tier approach that combines modified P-II kinetics with inverse modelling using TOXSWA v1.2 to derive reliable, compartment-specific P-II endpoints. The approach focuses on a) Determining separate DT50 values for the water and the sediment compartments; b) Accounting for adsorption through a physically consistent coupling factor that integrates sediment dry bulk density, distribution coefficient in sediment, and sediment porosity; and c) Reducing high compartment correlations.

To support this approach, a Python-based tool was developed to perform P-II level analysis (both standard and higher tier). The system solves ordinary differential equations (ODEs) as initial-value problems and minimise an ordinary least-squares (OLS) objective function using SciPy modules. The initial masses in water and sediment can be either fixed or fitted during the optimization process. Statistical parameters are handled as recommended by FOCUS degradation kinetics (2014), i.e. Chi2 is calculated, t-test is applied, and the confidence intervals are reported. The Fsed test is no longer a constraint using the modified P-II level approach. Results from the tool were validated against the widely accepted tool KinGUI for standard assessments.

Together with extrapolated (OECD 106) or optimised distribution coefficients (inverse TOXSWA) for individual sediments, the tool enables high-precision P-II level modelling for laboratory water/ sediment studies. As the higher-tier approaches are outlined in the appendices of FOCUS degradation kinetics (2014), the authors consider the described approach as an acceptable refinement in the EU risk assessment of PPPs.

3.10.P-Tu256 Identifying Model Parameter Hierarchies in FOCUS STEP 3

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To standardize the role of exposure 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.

This work applies global sensitivity analysis using Sobol' indices to identify influential parameters in FOCUS STEP 3. Parameters are sampled using low-discrepancy sequences and first-order and higher-order Sobol' indices are computed to quantify main and interaction effects. STEP 3 integrates TOXSWA to calculate surface water concentrations based on pesticide inputs from drift and runoff (via PRZM) or drainage (via MACRO), across three water body types and ten representative European scenarios reflecting climatic and pedological diversity.

Both substance properties and scenario descriptors are varied to map regions of parameter space where model outputs are most sensitive. The results show that sediment-related parameters have negligible influence on predicted environmental concentrations in surface water, indicating that the sediment compartment is not a driver of exposure in STEP 3. Among substance properties, the organic carbon–water partition coefficient (K_{oc}) emerges as the only consistently influential parameter, whereas degradation half-lives are largely subdominant across scenarios. This hierarchy implies that uncertainty reduction efforts targeted at K_{oc} yield the greatest improvement in prediction reliability. For less sensitive parameters central default values may be sufficient for less sensitive properties.

Disclaimer/Disclosure:

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3.10.P-Tu257 VERBER, a Catchment Scale Pesticide Risk Assessment Model for Surface Waters in Sweden

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Water authorities in European countries regularly need to carry out impact assessments of pesticides in their water bodies under the framework of the EU Water Directive (Directive 2000/60/EC). This requirement poses a challenge for Swedish water authorities since Sweden has up to 100,000 lakes and more than 500,000 kilometres of water courses. To support their work under the EU Water Framework Directive, we developed VERBER for surface waters, a pesticide risk management model. VERBER predicts average concentrations of active substances originating from arable fields at the outlet of sub-catchments in inter-connected networks of lakes and water-courses in Sweden. The model is intended to be used as a regional and national-scale screening-level tool to identify surface waters at risk of being affected by pesticide concentrations that pose a chronic toxicity to aquatic organisms or non-compliance with drinking water standards, and which therefore require extra attention such as monitoring.

The model calculates surface water pesticide concentrations by combining a parsimonious steady-state mass balance model with a classification tree trained on Swedish environmental monitoring data, to estimate the fraction of an applied pesticide lost to surface waters.

A comparison between average surface water concentrations predicted by VERBER and measured concentrations indicated that the model is generally realistic, but conservative in a limited number of situations.

Reference

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

3.10.P-Tu258 A Framework for Coupling SWAT+ Watershed Models with High-Resolution Stream Networks in xAQUATIC

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The xLandscape modeling framework enables spatiotemporally simulation of pesticide fate and effects at landscape scales for regulatory risk assessment. It provides core technical functions to run multiple consecutive environmental simulation models with pre-defined scenario data (e.g., land use and cover, hydrography,

topography, crop management). xAquatic is the aquatic component of xLandscape integrating various environmental exposure and effect models. However, developing high-resolution hydrological inputs for stream networks is resource-intensive. At the same time, numerous calibrated watershed models exist, yet they often lack the resolution required for spatially explicit landscape-scale aquatic risk assessment, such as single stream segments at approximate 100 m resolution and information on edge-of-field water bodies. To address this challenge, we developed a framework that takes existing SWAT+ watershed-scale models as hydrological inputs for xAquatic scenario development, enabling high-resolution pesticide fate modeling in small streams and ditches that are critical for ecological risk assessment but typically fall below the resolution of watershed models. Integration was achieved by mapping high-resolution xAquatic reach segments to SWAT+ channels based on spatial intersection with SWAT+ landscape units. Reaches beyond SWAT+ boundaries are mapped to the SWAT+ outlet, allowing extrapolation outside the model domain. An established hydraulic scaling approach was used to estimate discharge in individual xAquatic reaches from SWAT+ channel outputs: $Q_{xAquatic} = Q_{SWAT} \times (L_{xAquatic}/L_{SWAT})^\beta$, where Q represents discharge, L is cumulative upstream length, and β is a scaling exponent accounting for nonlinear discharge-length relationships. Hydraulic parameters are calculated using the Manning equation with trapezoidal channel geometry from xAquatic reach properties. The framework was applied in the lowland Funne catchment (5,440 ha) in North Rhine-Westphalia, Germany, a predominantly agricultural basin with poorly draining clay-rich soils, low hydraulic gradients, and extensive artificial drainage. In total, 563 xAquatic stream segments were mapped to SWAT+ channels and daily outputs were transformed to hourly time steps. By enabling reuse of existing watershed models within the xLandscape architecture, this framework is intended to lower barriers for landscape-scale aquatic risk assessments and to support regulatory adoption.

3.10.P-Tu259 Mapping Crop Development as Function of Calendar Date Across the EU for Use in the EU Regulatory Framework

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EU regulations require Environmental Risk Assessments (ERA) for non-target organisms exposed to active substances in plant protection products. Accurate prediction of crop phenology across Europe is essential to improve these assessments. The presented work aims to enhance pesticide ERA by precisely mapping crop development stages (i.e., the commonly used BBCH crop growth stages) across the EU.

A harmonized database of over 1.2 million phenological observations was compiled from public sources (C2D2, PEP725, TEMPO) and literature. The C2D2 database provided broad spatial coverage, while PEP725 and TEMPO offered complementary data concentrated in Central Europe and France, respectively. To address data gaps and spatial variability, models were developed for predicting sowing dates and phenological stages. A temperature-based rule was used to predict sowing dates, supplemented by a median rule for regions strongly influenced by management practices, such as irrigated maize in Southern Europe. Crop development was simulated with the WOFOST phenology model, which describes growth using a dimensionless development stage (DVS) ranging from emergence (0.0) to maturity (2.0), accounting for temperature, photoperiod, and vernalization. Model parameters (TSUM1 and TSUM2) were calibrated using observed phenological data aggregated by agroecological zones with similar climate conditions. Intermediate BBCH stages were then linked to the DVS scale, enabling wall-to-wall predictions of development stages across a 10×10 km EU grid. After calibration, WOFOST accurately reproduced phenological development for eight major crops: spring barley, potato, sunflower, maize, winter wheat, winter rye, winter rapeseed, and winter barley. The model successfully linked observed BBCH stages to the DVS scale, capturing the expected variability and overlap among stages.

Some examples of the resulting high-resolution spatio-temporal predictions of crop growth stages will be shown. The predictions provide valuable inputs for refining pesticide risk assessments under EU regulation. This work supports more accurate estimation of pesticide application dates; improves predictions of off-target exposure, including spray drift deposition, and pollinator risks, by better characterizing crop development stages; and enhances scenario development for EU regulatory frameworks such as the FOCUS surface water models and bee-exposure scenarios. The phenology database will be released as open data.

Disclaimer/Disclosure:

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advance concepts and methodologies for assessing the exposure of terrestrial non-target organisms to plant protection products.

3.10.P-Tu260 Data Availability and Quality Considerations when Collating and Harmonising EU Environmental Monitoring Data for Regulatory Purposes

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Access to publicly sourced environmental monitoring data has expanded considerably over the past twenty years, largely driven by open-data policies and the requirements of the Public Access to Environmental Information Directive (2003/4/EC). These datasets are generated by a range of organisations—national and regional environment agencies, water suppliers and other public-interest bodies—each collecting samples for different regulatory, operational, or research purposes. As a result, data originate from multiple sources, collected for varying aims and with differing methodologies, making the development of a harmonised, pan-European database complex. Yet this type of integration is essential for regulatory purposes, such as evaluating chemical quality across environmental compartments within the framework of the Plant Protection Products Regulation the Water Framework Directive.

The format of online accessible chemical monitoring data varies widely—from PDFs, spreadsheets, compressed archives, to sophisticated web portals that support query functions and visualisation tools. In other cases, data must be formally requested, and access may involve administrative steps or fees. While a substantial portion is available under open-data or permissive licences, some datasets containing third-party contributions are governed by stricter use agreements. Spatial data on monitoring locations are provided in different coordinate systems and with varying degrees of positional accuracy. The heterogeneity of formats, structures and conventions means that substantial processing is required before these data can be assembled into a coherent database suitable for regulatory evaluation.

This poster outlines data availability and quality challenges in collating and harmonising environmental monitoring data using real-world examples extracted from the EnviroDataMonitor data collection and curation service B1.0.5 database and the associated Chemical Harmonisation Tool which includes >500K substance synonyms trained to facilitate this harmonisation. The work presented will highlight emerging patterns and practical considerations relevant to large-scale chemical data integration, while also identifying gaps that need to be addressed to support future regulatory assessments. This ongoing effort aims to stimulate discussion on best practices and encourage collaboration toward more consistent and interoperable environmental monitoring datasets across Europe.

3.10.P-Tu261 Inventory and Characterisation of Chemical Monitoring Data across the EU

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Access to environmental monitoring data has expanded significantly across the EU in recent decades, largely driven by open-data policy developments and the increasing need for transparency in environmental decision-making. National and regional authorities (environment agencies, geological surveys, health departments) and water utilities routinely collect chemical quality data to meet diverse regulatory and operational objectives, following different sampling protocols, analytical methods and reporting standards. Consequently, although large volumes of information exist, they vary widely in quality, scope, structure and accessibility.

The assessment presented here draws on raw unaggregated chemical monitoring data gathered from countries across the EU and harmonised using the EnviroDataMonitor framework and the resulting b1.0.5 database. The work involved cataloguing monitoring providers and data sources, evaluating access routes and update frequencies, as well as characterising dataset formats, licensing conditions, and metadata completeness. Chemical and environmental dimensions were also considered, including the range of chemical classes monitored, spatial and temporal coverage, sampling matrices, analytical methods, and environmental compartments represented. Taken together, this provides an emerging evidence base on the scale, diversity and complexity of chemical monitoring information currently readily available across Europe, and the practical implications for large-scale data collation and harmonisation.

This poster presents emerging observations on the landscape of chemical monitoring data across the EU, highlighting common challenges to harmonisation and opportunities for improved data interoperability. Preliminary insights illustrate the diversity of data structures and access models encountered, and the need for coordinated approaches to make these datasets more usable for regulatory and scientific applications. The work

aims to support ongoing discussions around best practices and to lay the groundwork for more systematic data integration efforts in the future.

3.10.P-Tu262 Assessment of Pesticide Contamination in Dust from Quebec Residences based on a Method Adapted to Large-Scale Studies

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Household dust is a complex matrix that can accumulate a wide range of contaminants, including pesticides. Under indoor conditions, pesticide degradation is significantly limited by the absence of UV radiation, rainfall, and temperature fluctuations, leading to their persistence. Because people spend, on average, 90% of their lives indoors, household dust can be an important source of exposure. As global pesticide use continues to rise, there is a growing need for scalable approaches to assess pesticide contamination. This study aims to develop a contamination-free, simple, and representative sampling technique, combined with effective extraction and sensitive analysis, for quantifying pesticides in household dust, which could be easily implemented for large-scale epidemiological studies.

Dust samples were collected over 2-5 months, during the peak period of pesticide application, using Petri dishes placed inside participants' houses (n = 173) in an intensive agricultural region of southern Québec. A target list of 51 pesticides, including 18 fungicides, 19 herbicides, and 14 insecticides, was established based on their toxicity and regional use. After the accumulation period, dust was retrieved from the Petri dishes using cellulose wipes pre-moistened with isopropanol. QuEChERS was employed to quantitatively extract the pesticides from the samples, and liquid chromatography-triple quadrupole mass spectrometry was used for the analysis. This method was validated in accordance with the SANTE/11312/2021 guidelines.

Results showed that more than 97% of houses had at least one contaminant detected, and up to 17 pesticides were found in a single house. In total, 35 analytes out of 51 were detected in at least 1 Petri dish, with detection frequencies ranging from 1.3 to 69%. Fungicides were the most frequently detected pesticide class, with 16 out of 18 compounds found. Individual pesticide levels varied widely between homes, ranging from 5.9e-4 to 1.3e2 pg/cm²/day. The most frequently quantified compounds with the highest amounts were insecticides, including cis- and trans-permethrin, found in 32% and 60% of houses, respectively.

The present method provides accessible sampling and sensitive analysis suitable for large-scale studies. Furthermore, this is the first study to assess pesticide occurrence in indoor dust from residences near agricultural fields in Québec, and it will allow us to evaluate the significance of exposure to pesticides through dust.

3.10.P-Tu263 Monitoring of airborne pesticide transport and deposition in selected sampling sites in Germany between March and September 2025

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Airborne pesticide transport and deposition, e.g. from agricultural applications, are sparsely investigated sources of environmental contamination with potential ecotoxicological relevance for off-target organisms. To understand and quantify the entry of airborne pesticides into the environment, a monitoring campaign was performed in 2025 with a total of six sampling intervals of 4 weeks each. Pesticide concentrations in the air, as well as deposition were determined at three sites in different regions in Germany. Active air samplers were used to sample particulate matter with potentially adhering pesticides (using glass fibre filters) and volatile pesticides (using polyurethane foams). Deposition was determined using a bulk sampler to collect precipitation and dust. Samples were analysed by validated liquid- and gas-chromatography-mass spectrometry methods with limits of quantification in the low ng/L range (deposition) or in the pg/m³ range (air-concentrations). Additional experiments were performed to determine stability of the pesticides under sampling conditions for all three sample types, as well as breakthrough behaviour of the active air sampler.

Monitoring results showed trends in air concentrations and deposition of several pesticides over the sampling periods, which correlated with their typical application periods, as well as significant differences between sampling sites. Of the investigated pesticides and metabolites, dimethenamid, flufenacet, fluopyram, pendimethalin, prosulfocarb, prothioconazol-destio, terbuthylazine (and terbuthylazine-desethyl), as well as S-metolachlor were most frequently detected active substances/metabolites in these samples. The stability experiments for all samplers and breakthrough test for the active air sampler showed overall good recovery under sampling conditions. However, for individual pesticides there were significant losses by degradation, adsorption to the bulk sampler or breakthrough in the active air sampler. Overall, the results showed detectable pesticide entries into the environment potentially caused by long-distance air transport and subsequent deposition. These results may be used for a better understanding of long-distance transport and deposition of pesticides. The extent to which the results can contribute to evaluate the potential ecotoxicological risk of these pesticide entries to non-target organisms was not subject of this study and needs to be further investigated.

3.10.P-Tu264 Overcoming Phytotoxicity in Honey Studies: Insights from past Herbicide Tunnel Trials

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To establish Maximum Residue Levels (MRLs) for herbicides in honey, tunnel residue trials are the preferred methodology over open-field studies, as they ensure bees forage exclusively on the treated crop. This presentation outlines current approaches based on the SANTE 2016 guideline, specifically addressing the unique challenges of herbicide testing where the guidance left some questions open.

Unlike insecticides or fungicides, herbicides present a risk of phytotoxicity which can arrest nectar production. Consequently, trials must utilize sturdy surrogate crops. Interestingly, Phacelia, winter oilseed rape, and buckwheat have proven more robust for testing than expected, even when treated at full critical Good Agricultural Practice (cGAP) rates during flowering (BBCH 63-65).

We present data from 97 trials conducted across Central and Southern Europe. Results indicate that despite potential herbicidal effects on plant health, flowers often remain sufficiently attractive to sustain foraging. Daily monitoring of flight activity proved vital for determining the optimal sampling window, typically 7 to 10 days after application. The study confirms that valid honey samples, with sugar content and weights comparable to untreated controls, can be consistently obtained.

3.10.P-Tu265 Method Development and Determination of Pesticides in Residential Soils to Assess Human Exposure in Rural Areas of Québec, Canada

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Pesticides are widely used to protect crops but can contaminate air, water, and soil. Populations in agricultural regions are thus more likely to be exposed to pesticides than populations in urban centers, potentially increasing the risk of acute health effects. Despite these concerns, no study has yet assessed pesticide exposure pathways in communities near agricultural fields in Québec nor Canada. Among the multiple sources of pesticides, soils are particularly important since they can act also as reservoirs that can expose populations directly by dermal contact or accidental ingestion of soil, or through air particles formed by wind erosion.

This work aims to develop an analytical method to characterize pesticide occurrence in residential soils in Montérégie, Québec's most intensive agricultural zone. Residential soil samples were collected from 78 households within 1500m of agricultural fields in the summer of 2024. We targeted a total of 49 pesticides (18 fungicides, 17 herbicides, and 14 insecticides), selected according to their use in the region and human toxicity. After collection, samples were stored at -20°C. Prior to extraction, they were air-dried for 24 hours and sieved to 2 mm. A QuEChERS extraction technique was employed, and multiple parameters (solvent ratio, salt ratio, sorbent ratio, etc.) were optimized using Design of Experiments based on the Plackett-Burman approach. Pesticide contamination was then quantified using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Method performance was evaluated according to SANTE/11312/2021/v2 international standard, where linearity ($R^2 > 0.99$ and $\leq 20\%$ deviation), precision (%RSD 1-7%), trueness (-13.58-13.46%), and LOQs (1-25 ng/g) fulfilled the acceptance criteria defined in the guideline.

Preliminary results (39 out of 78 samples) show the presence of multiple pesticides, mainly atrazine, chlorantraniliprole, imidacloprid and metolachlor at concentrations ranging from 1 to 11 ng/g. As for clothianidin, it was detected at concentrations up to 24 ng/g. Detection frequencies, number of pesticides and cumulative concentrations will be presented.

This work provides a validated analytical approach and baseline data on residential soil contamination near agricultural areas, supporting future assessments of pesticide exposure and associated health risks in rural populations.

3.10.P-Tu266 Pesticides in private and community gardens

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While the soil quality, including contamination, is carefully monitored in agricultural land, the soils found in urban environments are almost entirely overlooked. At present, there is no regulation that would require the regular monitoring of urban soils or any systematic assessment of the potential risks they may pose to human health as a result of chemical or historical contamination. Yet many urban citizens who own small private gardens use them to grow fruits, vegetables, herbs, and ornamental plants. These gardeners often assume that home-grown produce is fresher, safer, and generally healthier than comparable goods purchased in supermarkets. However, this expectation may not always reflect reality. Urban soils, including those in private gardens, can suffer from reduced quality due to their complex history, previous land use, or long-term exposure to pollution sources such as traffic emissions, industrial activities, waste disposal, and the accumulation of legacy contaminants. Consequently, the produce grown in such soils may carry risks that gardeners are unlikely to be aware of.

For these reasons, we aimed to determine the overall soil quality, with particular emphasis on pesticide contamination, in typical city gardens in the Czech Republic and to discuss both the likely origins of such contamination and its potential environmental and health consequences. In total, the study includes more than 80 gardens ranging in size from 70 to 1400 m². The selected gardens are situated either adjacent to family houses or within traditional gardening colonies that are common in many Czech cities. The soils in these locations can be classified as technosols, chernozems, or fluvisols—typically loamy soils formed under a continental climate. By examining a diverse set of garden sites and soil types, the study seeks to provide an evidence-based understanding of the current state of urban garden soils and highlight the need for more systematic monitoring policies in the future.

The results indicate relatively high concentrations of p,p'-DDE (more than 0.1 mg/kgsoil in 21 cases, with concentrations exceeding 1 mg/kgsoil in 3 cases) and also significant concentrations of p,p'-DDT in some samples (more than 0.1 mg/kg soil in 7 cases). From the currently used pesticides, the highest concentration was found for azoxystrobin (more than 0.1 µg/kgsoil).

Disclaimer/Disclosure:

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3.10.P-Tu267 A triad approach to assess the cocktail effect in surface water: a case study on the Mehaigne River (Wallonia, Belgium).

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Current monitoring of the chemical status of water bodies fail to characterize the likelihood that complex mixtures of chemicals affect water quality. In order to assess the risk induced by cocktail effect in Belgian surface waters, a transect of the Mehaigne River, which is under pressure from agriculture and untreated urban wastewater, was studied using a triad approach comprising the use of chemical analyses (100 parameters), biomarkers (feeding rate, acetylcholinesterase (AChE) inhibition, Glutathion-S-Transferase (GST) induction) and bioindication (macroinvertebrates standardized global biotic index; biological, physiological and ecological traits; and SPEAR pesticides index).

Within this framework, five caging campaigns using the amphipod *Gammarus pulex* were conducted at five sites along the Mehaigne, as well as at one reference site (Blanc-Gravier, Colonster).

An inhibition of AChE activity (>25%) was observed for two sites, one upstream in the agricultural sector, and one downstream in the urbanized sector, which suggests the presence of insecticides in the river though chemical analyses were not particularly correlated. GST activity was induced (>20%) in two sites (not statistically significant). A reduction of feeding rate was observed from upstream to downstream, showing a global degradation of water quality.

The integration of the three components (chemistry/bioassays/bioindication) allows to clarify the cause-effect relationships at each site, with a very degraded situation in the area close to the source, which is affected by toxic pressure linked to pesticides. The situation remains very degraded throughout the upstream sector. A clear improvement in the biogenic quality of the environment (microhabitats, organic pollution, eutrophication) is then observed, but the SPEAR index still indicates a significant impact related to pesticides, which is confirmed by the detection of herbicides, among other, at all the sites. Downstream, all indicators are pointing towards deterioration, which is linked to a strong urbanization of this sector promoting a supply of waste waters.

EBM tools thus constitute an interesting approach to highlight the cocktail effect of pesticides, particularly when associated with complementary approaches, and to characterize more precisely the anthropogenic pressures exerted on the river. The development of additional biomarkers associated to other modes of actions would contribute to improve this triad approach.

3.10.P-Tu268 Insecticide Bans Drive Declines in Aquatic Toxicity: Insights from 16 Years of Monitoring in Sweden

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Over the past decade, several insecticides have been banned within the EU with the aim of reducing risks to human health, protecting particularly vulnerable species and ecosystems and preventing long-term environmental contamination.

The aim of this study was to evaluate how these regulatory measures have influenced insecticide concentrations and toxicity in agricultural streams, using data from the Swedish national monitoring program for pesticides. Since 2002, the monitoring program has conducted high-frequency surface water sampling in four agricultural streams in southern Sweden. In parallel, farmers in the catchment areas are interviewed about their crop production and pesticide use.

Analyses of more than 180 substances between 2009 and 2024 show that insecticides, although typically present at lower concentrations than fungicides and herbicides, account for the largest share of toxicity in the streams. However, the long-term trend is clear: both insecticide use and the measured concentrations in surface water have declined markedly. This is also reflected in a decrease in overall toxicity in the watercourses, although some still approved insecticides — such as the pyrethroid tau-fluvalinate and the neonicotinoid acetamiprid — have increased in both use and measured concentrations following the bans.

Overall, the results show that the bans have had the intended effect. The use of many highly toxic insecticides has decreased or ceased, which is reflected in lower concentrations and reduced toxicity in the environment. This serves as a concrete example of how regulatory measures can lead to positive environmental outcomes.

3.10.P-Tu269 Collection of Sediment Data in Small Edge-of-field Watercourses and Ponds at the EU-FOCUS Scenarios Locations for use in Pesticide Registration

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In the EU the pesticides exposure to assess risks for aquatic organisms is calculated using models and scenarios. The so-called FOCUS scenarios include fifteen small edge-of-field water bodies, ditches, streams and ponds, that represent the major EU agricultural areas. Currently the sediment of all scenarios is characterized by the same properties, including a relatively low value for the organic carbon content of 5%. This low value leads to a conservative exposure in the water layer, thus a conservative risk assessment for aquatic organisms. However, it also leads to an underestimation of the exposure in sediment and thus it may underestimate risks for sediment-dwelling organisms. EFSA granted a project for a literature search and sampling campaign to collect data on sediment properties of freshwater bodies in the major agricultural areas of the European Union (project EFSA/2025/OP/0005, ongoing). The ultimate goal is to improve the sediment descriptions of the EU-FOCUS surface water scenarios to improve the quality of the aquatic risk assessment.

Locations of water bodies were selected in the vicinity (i.e., 30 km) of the existing scenario location coordinates using a waterbody map of the EESE project (contract OC/EFSA/PREV/2023/02), arable crop areas from Corine Land Cover and European Union Crop type map of 2022, and the FOCUS maps for the areas represented by the scenario. The sampling campaign took place in the spring season of each of the three regulatory zones, between April 2025 and March 2026. The sediment of 30 water bodies; 8 ditches, 16 streams and 6 ponds, was sampled. Following a randomized sampling scheme 20 cores were taken in a stretch of 100 m for ditches and streams and scaled to size for the ponds. From each core 0-2, 2-5 and 5-10-cm layers were sampled. Determined were bulk density, porosity and organic matter content. For the 5-10-cm layer also the granular composition was determined, classifying the sediment within the USDA soil classification system.

The results show, that the variation in bulk density and organic matter content within and between water bodies is very large. Examples for a pond and a stream are presented. On the basis of these data the sediment properties in the current EU-FOCUS surface water scenarios can be improved to promote a more realistic risk assessment for benthic organisms and rooted water plants in the registration procedure at EU level.

3.10.P-Tu270 Spatial analysis of vulnerability of agricultural lands to surface runoff in Europe

Penélope González Galarza, **Sebastian Multsch** and Tom Schroeder, BASF, Germany

The authorization of plant protection products requires an environmental risk assessment to evaluate potential adverse effects on non-target organisms. For aquatic organisms, risk assessment considers three main exposure pathways: runoff, drift and drainage into edge-of-field waterbodies. The FOCUS (FORum for the Co-ordination of pesticide fate models and their USE) surface water guidance provides a conceptual framework for estimating environmental exposure using simulation models, parameterization assumptions, and representative European scenarios. The FOCUS surface water scenarios (R1–R4) assume specific slope ranges (R1: 2–4%, R2: 10–15%, R3/R4: 4–10%) intended to represent realistic worst-case conditions.

This study applied geospatial analysis to quantify the extent to which actual crop fields near water bodies in the EU align with FOCUS slope assumptions. Spatial datasets of crop distribution and field slopes in the proximity of water bodies (50 m and 100 m buffers) were integrated to calculate overlap between FOCUS slopes and real-world conditions.

Results indicate that the proportion of crop area meeting both proximity (<50 m) and slope (>4%) criteria is extremely low—typically <0.4%. This demonstrates that FOCUS slopes represent only a small fraction of actual field situations adjacent to water bodies. Consequently, Tier 1 assessments based solely on these scenarios may overestimate real-world runoff exposure. Incorporating actual slope distributions into risk characterization enables more proportionate mitigation strategies.

The findings support slope-based label language (e.g., “Do not apply on soils with slope >4%”) as a targeted measure for the limited subset of fields with slopes similar to FOCUS assumptions.

3.10.P-Tu271 Comparison of FOCUS Step 4 simulations using the updated SWAN-VFSMOD scenarios with standard fixed reduction efficiencies according to FOCUS Landscape

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The standardized FOCUS approach for pesticide exposure assessment in surface waters (FOCUS_{sw}) consists of four steps. Mitigation of surface runoff inputs with vegetative filter strips (VFS) can be assessed in Step 4 using the tool SWAN, which implements both fixed VFS efficiencies according to the FOCUS Landscape report and an option to apply runoff mitigation using the mechanistic, event-based model VFSSMOD. Recently a set of updated SWAN-VFSSMOD scenarios for FOCUS Step 4 has been derived. The updated scenarios are both more realistic and more protective than the previous ones and will be included in the upcoming SWAN release (v.5.3).

The objective of this study was to compare the updated SWAN-VFSSMOD scenarios with the standard fixed reduction efficiencies from FOCUS Landscape in terms of resulting exposure in surface water. For this aim, a set of FOCUS Step 4 simulations ($n = 216$) were performed (6 compounds, 2 crops, 2 application timings per crop, 2 values of VFS length in flow direction). The simulations were conducted for the two different approaches: i) “new”: new SWAN-VFSSMOD scenarios, using VFSSMOD v.4.5.2), ii) “old”: current standard approach with fixed efficiencies. Simulations were run over 20 years. Several target variables were analysed: i) pesticide load reduction efficiency (?P) for each event; ii) Predicted Environmental Concentrations (PEC_{sw}) for each event; iii) global PEC_{sw,max} for the 20-year period; iv) PEC_{sw,max} for the original 12-month period used in FOCUS_{sw}; and v) ranked annual PEC_{sw,max} and PEC_{sed,max}.

For the performed 216 simulations, the new approach yielded predominantly higher pesticide trapping and lower PEC than the old one. However, for the scenario / water body combination R1 pond (and partly R1 stream) combined with the highest simulated K_{foc} (1000 L kg⁻¹), the calculated PEC_{sw}, global PEC_{sw,max} and high ranks of annual PEC_{sw,max} and PEC_{sed,max} tended to be higher with the new approach. However, due to the limited range of sorption and degradation parameter values investigated, the results cannot be generalized to the whole substance parameter space for pesticides.

In summary, compared with the current standard fixed efficiency approach, the new approach with the updated SWAN-VFSSMOD scenarios yielded predominantly less conservative, but sometimes also more conservative ?P and PEC, reflecting the increase in realism and accuracy (and thus reduction of uncertainty) due to the use of a mechanistic, event-based model.

3.10.P-Tu272 Challenges in applying the joint ECHA & EFSA water treatment guidance document for plant protection products

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The joint ECHA & EFSA guidance document (GD) on the impact of water treatment processes on residues of active substances, or transformation products, in water used for drinking water, was approved in July 2023 and comes into force in April 2026. This poster takes a step-by-step approach of applying the guidance to an example plant protection product with transformation products, highlighting the challenges of applying the GD. Difficulties and compromises of simulating metabolites at FOCUS Step 3 for surface water are highlighted, along with the use and derivation of zonal dilution factors and the exposure scenarios. Suggestions for changes to the process will be made for both ground and surface water.

3.10.P-Tu273 Integrating slow-release behaviour of encapsulated plant protection products into EU regulatory leaching assessment: Current knowledge and regulatory challenges

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Plant protection products (PPPs) encapsulated in a polymer matrix, known as capsule suspension (CS) formulations can improve the functionality of active ingredients and alter product risk profiles. While CS formulations are mostly known for better targeting of pest species, increased efficacy and lower application rates, emerging evidence indicates that CS formulations also exhibit slow-release characteristics, influencing environmental fate and potentially mitigating leaching risks of PPPs. However, current EU regulatory frameworks for groundwater exposure assessment do not explicitly account for such controlled-release behaviour. To address this gap, we conducted a comprehensive review of peer-reviewed literature and EU regulatory dossiers to evaluate how CS formulation properties affect leaching potential of PPPs compared to other formulations. Our analysis focused on soil mobility, degradation kinetics and derivable release kinetics from scientific and regulatory studies conducted under realistic soil conditions. As existing leaching assessments lack parameters for slow-release kinetics, we also explored options to incorporate slow-release kinetics of CS formulations into the existing EU groundwater modelling framework to enable more realistic exposure predictions. Overall, we aimed to synthesize knowledge on the impact of slow-release behaviour of CS formulations for groundwater exposure and raise regulatory awareness that current leaching assessments may

overestimate groundwater risk of CS-formulated PPPs. Incorporating formulation-specific release behaviour into regulatory modelling could enable more realistic exposure predictions and encourage innovation in sustainable PPP formulation technologies.

3.10.P-Tu274 Bringing Rice Cultivation Practices into Environmental Risk Assessment

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Environmental risk assessment (ERA) for pesticides in rice has historically relied on the MedRice guidance (Sanco/1090/2000 – rev.1; 2003), which assumes a static, flooded field scenario. This regulatory approach does not capture the diversity of European rice cultivation, where herbicide applications are often made to drained or semi-drained fields and water management involves multiple, dynamic flooding and drying cycles. As a result, current regulatory scenarios may miscalculate environmental exposures, potentially leading to inappropriate levels of protection for aquatic and terrestrial ecosystems.

Our study critically evaluates both the current estimates. We further assess the value of adopting physically-based models, such as RiceWQ, and the development of standardized, regionally representative scenarios. Comparative simulations were conducted using the MedRice tool and RiceWQ, parameterized with both MedRice scenarios (clay and sand soils) and new ICPS-defined country-specific scenarios (Update and harmonization of rice pesticide risk assessment and revision of European guidelines: A proposal; 2018), across major rice-growing regions including Italy, Spain, France, Greece, and Portugal. Predicted environmental concentrations (PECs) for surface water were compared to highlight the impact of scenario and model selection.

Our results show that the MedRice default scenario fails to reflect the variability of real-world practices, often leading to inaccurate exposure predictions. While the draft guidance introduces improvements—such as options for dry applications and new scenarios—it still falls short in representing regional agronomic realities. In contrast, RiceWQ, when parameterized with country-specific scenarios, yields more realistic exposure estimates.

These findings underscore the need for ERA in rice to transition toward the routine use of physically-based models like RiceWQ, supported by harmonized, regionally relevant scenarios. Enhanced collaboration among regulators, industry, and researchers will be essential to ensure scientifically robust and practically relevant risk assessments, ultimately improving both regulatory acceptance and environmental protection in rice cultivation.

3.10.P-Tu275 French Regulatory Feedback on EFSA Guidance for Predicting Environmental Concentration of Plant Protection Products in Soil: Impact Assessment of Variations in Substance-Specific Input Parameters

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The European Food Safety Authority (EFSA) has released a guidance (EFSA, 2017a) providing the methodology for calculating the predicted environmental concentrations of active substances and transformation products in soil (PECsoil) which can be used to conduct terrestrial risk assessments in the context of the Regulation (EC) No 1107/2009. This guidance on PECsoil calculations aims to ensure a more robust estimation of the exposure levels for soil organisms following application of Plant Protection Product (PPP). The methodology relies on specific soil scenarios which take into account knowledge on soil, weather and crop. The PECsoil calculations for the active substance and/or its metabolite(s) require only a few input parameters mainly related to (a) the application conditions of PPP (e.g., crop, application rate, crop growth stage, application method) and (b) the properties of the active substance/metabolite(s) (e.g., soil degradation and soil mobility). To facilitate the calculations, the analytical PERSAM tool has been released in addition to updated versions of the existing FOCUS numerical tools.

This work focuses on how variations in several input parameters related to the assessed compound (especially soil degradation half-life, DT50 and soil mobility, Koc) affect PECsoil estimates under the EFSA methodology. PECsoil were calculated for several combinations of input parameters (DT50/Koc) and different types of crops (annual and perennial), based on the recommendations of this guidance.

This work presents a first feedback on the sensitivity of the models against the selected input parameters in

order to better anticipate its use in risk assessment.
a) EFSA Journal 2017;15(10):4982

3.10.P-Tu276 Environmental Safety Considerations in EFSA's Statement of Chitosan and Chitosan Hydrochloride for Plant Protection Use

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The use of natural-based substances to improve crop quality and consequently food production was extensively developed during recent years as one of the promising approaches for more sustainable agricultural practices. Chitosan and chitosan hydrochloride, thanks to their eliciting properties, gained considerable interest in use as pesticides besides the other agricultural uses such as soil amendments or fertilisers. Both substances are approved in the EU as basic substances under certain conditions of application.

In January 2024, the European Commission asked EFSA for an opinion regarding the review of approval of chitosan and chitosan hydrochloride as per Article 23(6) of Regulation 1107/2009. In this regulatory context, it was requested to investigate whether the estimated levels of chitosan and chitosan hydrochloride resulting from the approved uses as basic substance would exceed the levels expected to occur in the environment, either naturally or derived from other uses, and consequently whether there is a need to advise on their safety to non-target species occurring in the impacted environmental compartments. To this purpose, the EFSA Panel on Plant Protection Products and their Residues (PPR) adopted a statement addressing such request accompanied by an EFSA Scientific Report outlining an estimation of the natural chitin presence in an average agricultural soil.

This poster highlights the main challenges encountered in estimating the natural levels of chitosan in the environment, starting from an assessment of its precursor chitin, and considering the rate of degradation of chitosan, strongly dependent on microbial conditions and its chemical properties. This estimation was based on the available scientific literature and derived from a polynomial function. The obtained estimation was then compared against the Predicted Environmental Concentrations of chitosan and chitosan hydrochloride calculated according to their approved uses as basic substances and the EU regulatory practices. The comparison was carried out in a quantitative and a (semi)quantitative way for the soil and freshwater compartments, respectively. Results based on the available information in ecotoxicology and environmental fate properties of chitosan, concluded that exposure levels resulting from the use of chitosan and chitosan hydrochloride as basic substance are not considered sufficient to trigger any risk for non-target organisms.

3.10.P-Tu277 Drinking Water Residue Testing: The Validation of A Positive Control Substance to Support Water Treatment Studies

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The safety of an active substance is evaluated under Regulation (EC) No 1107/2009 and considers impact on the environment, animal and human health. In recent years, a data gap has emerged regarding the influence of water treatment processes on residues of active substances. This concern has led to the issuance of new guidance by the European Food Safety Authority (EFSA) and European Chemicals Agency (ECHA) on assessing the impact of water treatment processes (e.g., chlorination, ozonation) on active substances and their environmental transformation products. The guidance document sets out a tiered approach to determine if harmful substances may be formed during treatment of ground or surface water abstracted for drinking water. It is crucial that experimental methods used to determine if any treatment transformation products are formed, are robust and reproducible. A positive control substance can be useful to demonstrate the reliability of experimental methods and give confidence to data presented in regulatory studies. This presentation provides the results of screening to find, and validation of a suitable substance that can act as a positive control under all water treatment processes described in the guidance document. The validation study includes the experimental workflow, analysis of CIPAC-C water (with dissolved organic carbon) treated at a low concentration with generic compounds and subsequent stability assessments using sensitive LC-MS/MS. Also included, is the validation of a quality control procedure to demonstrate the suitability of a chromatographic method used for non-targeted screening and identification of transformation products.

3.10.P-Tu278 Novel 8-µl Blood Micro Sampling Followed by LC-MS and GC-MS Analysis Shows Low Levels of Pesticides in Farmland Birds from Sweden

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Bird declines in European farmland have been linked to pesticide-driven food depletion and chronic exposure to pesticides. Yet, to what extent birds are exposed remains largely unknown, partly due to the practical and ethical aspects of collecting blood from small, wild species, but also to the analytical challenges associated with trace level determination from small sample volumes.

The Capillary Microsampling (CMS) technique was used to collect and quantitatively handle 8 µl blood samples from nestling yellowhammers *Emberiza citrinella* (N=5), nestling skylarks *Alauda arvensis* (N=40) and adult ortolan buntings *Emberiza hortulana* (N=21). With CMS an exact sample volume is collected with end-to-end glass capillaries (here after penetrating the brachial vein with a cannula) and easily treated using ordinary liquid handling.

Preparation of blood samples for LC-MS/MS analysis included addition and mixing with internal standards (ISs) and protein precipitation using acetonitrile, to a final volume of 80 µl, thus a ten-time dilution. For GC-MS, the water-based blood-IS-mixture was instead prepared by miniaturized liquid/liquid extraction using a 1:1 mixture of cyclohexane:ethyl acetate (2 x 250 µl), followed by evaporation and reconstitution in 80 µl cyclohexane:acetone 9:1. To our knowledge, this is the first report of CMS being combined with liquid/liquid extraction and GC-MS analysis.

Despite the small sample volume, the LC-MS/MS multimethod (100 compounds) showed an average detection limit of 0.77 ng/ml (range 0.016-10 ng/ml). The GC-MS method was limited to early eluting, low-boiling compounds such as chlorpyrifos, due to the complex blood matrix. For high-boiling compounds like pyrethroids, the method lacked robustness and data quality. To improve results for these compounds, additional clean-up of extracts is needed.

Pesticide residues were detected in half of the nestlings with up to 7 pesticides in a single sample (average 2). Concentrations of individual pesticides were relatively low (0.02 to 50 ng/ml) and were restricted to fungicides (N=8) and herbicides (N=5) in nestlings. Most frequently detected were the two fungicides fenpropimorph and pyraclostrobin and the herbicide tribenuron-methyl.

Our results also show that exposure of migratory ortolan buntings to the insecticide chlorpyrifos outside of Sweden can be detected in blood samples collected on their Swedish breeding grounds (7/10 tested positive; 0.25 to 0.56 ng/ml).

Disclaimer/Disclosure:

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https://papers.ssrn.com/sol3/papers.cfm?abstract_id=5439852

3.10.P-Tu279 Neonicotinoid residues in migratory birds from a key stopover site in South Korea

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Neonicotinoids are widely used insecticides, and their effects on non-target species continue to raise concern. Migratory birds, which travel across diverse geographic regions and habitats, including areas with intensive pesticide use, are particularly vulnerable to exposure. Their long-distance movements and dependence on multiple stopover sites increase the chance of accumulating these chemicals from different sources, with possible consequences for their health and survival. Although the effects of neonicotinoids on invertebrates and some resident bird species are well studied, information on exposure levels in migratory birds remains limited. This study examined neonicotinoid exposure in migratory birds by measuring these compounds and their metabolites in different tissues. Feathers and muscle samples were collected and analyzed for nine neonicotinoids and eight metabolites using LC-MS/MS. Our results showed that neonicotinoid residues were present in the bird samples. Thiacloprid was the most frequently detected compound (40%), followed by acetamiprid-N-desmethyl (35%). The highest concentration was observed for nitenpyram at 14.9 ng/g, followed by thiacloprid at 2.46 ng/g. These results show that migratory birds encounter neonicotinoids during their annual cycle and highlight the potential ecological risks linked to the widespread use of these insecticides.

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3.10.P-Tu280 Integrating Chemical Diversity and Matrix Complexity: A Validated LC-MS/MS Multiresidue Framework for Realistic Exposure Assessment

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Realistic environmental exposure assessments require experimental designs capable of reflecting both the diversity of pesticide chemistries and the analytical complexity of agricultural matrices. In this study, we develop a comprehensive and integrative framework for evaluating a broad suite of active substances spanning multiple functional categories—including insecticides, herbicides, fungicides, plant growth regulators and safeners—and covering a wide spectrum of physicochemical profiles and modes of action. Incorporating such chemical diversity is essential to account for the range of behaviours that pesticides can exhibit once applied under field-relevant agricultural conditions, and to improve the realism of multi-residue exposure assessments.

To address the substantial variability encountered across crops, sample types were selected according to the classification logic described in SANTE/2020/12830. These include high-water-content commodities, acidic and sugar-rich materials, dry and cereal matrices, high-fat matrices, and difficult matrices such as roots and tubers. Organizing samples within this regulatory framework ensures that the study encompasses the matrix categories most commonly represented in monitoring programmes and ecological risk-assessment workflows, thereby improving the transferability and regulatory relevance of the generated data.

A harmonized LC-MS/MS multiresidue method is validated and optimized to address the diverse extraction challenges, co-extractive interferences and matrix effects associated with these SANTE-defined groups. Particular attention is dedicated to ensuring consistent analytical performance across contrasting pesticide classes and matrix types, allowing reliable, sensitive and comparable quantification of residues under realistic agricultural scenarios.

By integrating chemical breadth, matrix representativeness, validated methodology and alignment with regulatory guidance, this work establishes a robust foundation for evaluating cumulative exposure patterns in contemporary agro-ecosystems. The resulting framework is intended to support the development of more effective monitoring strategies, enhance exposure modelling efforts and contribute to more nuanced ecological risk assessments that account for the complexity of real-world multi-residue situations.

3.10.P-Tu281 METABOLOMIC ANALYSIS AND BIOCHEMICAL ANALYSES IN PLASMA, GONADS, AND LIVERS OF FISH EXPOSED TO BENTAZONE

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Bentazone is a selective herbicide, without systemic action, which is chemically a thiadiazine. It inhibits photosynthesis at Photosystem II, being effective in weed control. It is a herbicide widely used in irrigated rice crops, extensively employed in Southern Brazil. In this study, the possible deleterious effects of bentazone were evaluated in fish (*Oreochromis niloticus*), under laboratory conditions, exposed to concentrations of 170 µg/L. Fish were kept in groups of 10 individuals, control and exposed, in 300L tanks, fed with commercial feed. Blood, liver, and gonad samples were collected after a 10-day exposure period, with daily water renewal in the tanks. The obtained blood plasma was analyzed regarding total cholesterol, glucose, albumin, total proteins, AST, ALT, and alkaline phosphatase. Gonads and liver were homogenized, extracted with solvent (MTBE), and analyzed by GC/MS-TOF, in addition to the analysis of total carbohydrates and lipids. The results obtained indicated significant alterations in the levels of the enzymes ALT and alkaline phosphatase, and discrete alterations in AST. Total lipid and carbohydrate concentrations in the gonads and liver exhibited significant alterations, as well as alterations in the fatty acid, cholesterol, and carbohydrate profiles analyzed by GC/MS. A significant reduction in plasma glucose and cholesterol was observed. Total proteins and albumin did not present significant modifications. The fish exposure to bentazone provoked important metabolic alterations in the liver and gonads of the fish under study.

Keywords: bentazone, herbicide, toxicity, metabolomics, hepatic alteration.

3.10.P-Tu282 From sample to cell: Decoding Glyphosate exposure through and integrated platform

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Glyphosate is one of the most widely used herbicides worldwide, and its extensive application in Brazil, raises concern about human exposure through food and environmental matrices. Accurate quantification of glyphosate

and its main metabolite, aminomethylphosphonic acid (AMPA), is essential for supporting monitoring and regulatory actions. This study developed an analytical method for determining glyphosate and AMPA in grain samples, including soybean, bean, corn, and rice. Multivariate optimization approaches were performed to streamline method development, reducing time and resource consumption while maximizing recovery rates and minimizing matrix interference.

Quantification was performed using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Chromatographic analysis employed an InfinityLab Poroshell CS-C18 column (Agilent®) maintained at 40°C, while the autosampler was kept at 8°C, using a mobile phase consist of 0.1% of formic acid in water. Residue levels will be compared to the Reg/EU 239/2013, which is used in Brazil and define maximum residues levels (MRLs) of 20.00 mg/kg for soybean, 1 mg/kg for corn, 0.10 mg/kg for rice and beans.

To assess the potential health implications, quantified residues will undergo an in vitro bioaccessibility assay using a Caco-2 cell monolayer, followed by re-quantification of the permeated fraction. Additionally, glyphosate, AMPA, and selected commercial formulations are being evaluated in cytotoxicity (MTT), genotoxicity (comet assay), and micronucleus assays across a range of concentrations, including those near regulatory limits. Preliminary MTT results indicate higher toxicity for commercial formulations compared with isolated active ingredients, underscoring the importance of continuous monitoring and regulatory vigilance to ensure food safety. Integrating chemical quantification with biological response enables a more realistic assessment of potential health risks, supporting evidence-based regulatory decisions and strengthening food safety initiatives.

Disclaimer/Disclosure:

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3.10.P-Tu283 Optimized and Validated Novel SULLE-LC-MS/MS Method for the Determination of Neonicotinoids in Honey Samples

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Due to their high effectiveness, neonicotinoids (NQs) are among the most commonly used insecticides worldwide. These compounds have been repeatedly detected in aquatic environments, soil, and food, confirming their widespread distribution in ecosystems. Honeybees, thanks to their wide foraging range and continuous collection of nectar and pollen, enable the monitoring of environmental contamination. Honey can provide a matrix reflecting the accumulation of pollutants present in plants within a radius of several kilometres of the hive, making it a useful indicator of pollinator exposure to pesticides. Efficient extraction of NQs from a complex matrix such as honey remains a significant analytical challenge. Therefore, the development of sensitive and precise methods for their determination is crucial for a reliable assessment of pollinator exposure to these compounds.

In order to effectively monitor pollinator exposure to NQs, a novel method for determining residues of seven NQs in aqueous honey solutions was developed and validated. Sugaring-out liquid-liquid extraction (SULLE) with a hydrophobic modifier was used, and the analytes were determined using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The extraction conditions were fully statistically optimized to maximize the efficacy of the method. Extraction effectiveness was 61-86%, depending on the compound and its concentration. The developed procedure was validated using a real matrix. Limit of detection was equal to 0.17 ng/g for majority of the compounds.. The created method was used to determine the degree of contamination of 108 natural honey samples from different regions of the world. The most frequently detected compound was acetamiprid, present in 88% of the tested samples, and followed by thiacloprid detected in 54% of honeys. The results confirmed the widespread exposure of bees to NQs in the agricultural landscape.

The developed method is characterized by high precision, accuracy, and selectivity, ensuring reliable determination of NQs in honey. Compared to the standard QuEChERS method, the modified SULLE is faster to perform, less expensive and requires fewer sample-preparation steps, making it an effective alternative in the analysis of pesticide residues in food.

3.11.A Exploring the Chemical Exposome through Advanced Target and Nontarget Screening: Bridging Occurrence, Transformation Products, and Human Health

3.11.A.T-01 Advancing the Chemical Exposome: Integrated Targeted and Non-targeted HRMS Investigation of PFAS Occurrence and Transformation in the Rhône River

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Per- and polyfluoroalkyl substances (PFAS) constitute a vast group of anthropogenic fluorinated compounds exceeding 7 million entries currently listed in PubChem. Targeted analysis captures only a fraction of PFAS owing to the restricted availability of commercial standards. In the southern Lyon area (France), a region marked by intense anthropogenic pressures including a major chemical industrial activity that have led to PFAS contamination and national-scale crises, local authorities implemented monitoring actions in 2022 focusing on 22 compounds in Rhône River waters, groundwaters, sediments and fish. However, contamination may have been underestimated as many emerging compounds remain unidentified. Although regional sources are mainly linked to a single fluoropolymer factory, additional inputs may also contribute. In this context, advanced non-targeted screening analysis (NTS) based on high-resolution mass-spectrometry (HRMS) is crucial for detecting both known and unknown PFAS, thereby supporting source identification. To assess the magnitude of contamination and identify compounds relevant to source tracing, this study combined a targeted analysis of 50 PFAS with NTS on the same extracts and HRMS platform (Thermo Exploris 120). Sampling of fish, sediments and groundwater (2024 – 2025) covered a longitudinal gradient including the Lyon area, with emphasis on an industrial harbour affected by a major fire (1987) and long-term aqueous film forming foam (AFFF) use. Fish were mostly contaminated with legacy PFAS, while groundwater and sediments were contaminated with both legacy and emerging PFAS, such as fluorotelomer sulfonate (FTS) and fluorotelomer sulfonamidoalkyl betaine (FTAB). Notably, 6:2 FTAB was found at concentrations comparable or even higher than those of PFOS in these matrices. Industrial harbour sediments showed the highest levels, whereas upstream of Lyon displayed significantly lower concentrations. Preliminary NTS annotations in groundwater samples revealed additional unexpected PFAS compounds spanning several classes. Our results highlight the need to complement targeted methods with non-targeted screening to achieve a broader characterization of PFAS occurrence. Emerging PFAS were seldom detected in fish, yet biotic matrices remain key to assessing contaminant transfer from abiotic compartments. Future work will refine suspect identification and semi-quantitation using structure- and ionization-matched reference standards.

3.11.A.T-02 Non-targeted screening of environmental contaminants in passive air sampler extracts from Montreal using LC-Q-TOF-MS and GC-Q-TOF-MS.

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Air pollution is closely associated with increased lung cancer incidence and mortality. Because many semi-volatile industrial chemicals, pesticides and combustion by-products have been classified as endocrine disrupting or genotoxic, their mixtures in urban air, even at trace levels, could have cumulative effects on human health. However, evidence for the impact of outdoor air pollution on the risk of other types of cancer and chronic diseases, remains limited, in part because the composition of such mixtures in outdoor air remains poorly characterized. Non-targeted analysis (NTA) based on high resolution mass spectrometry (MS), allows for the comprehensive analysis of contaminants in environmental samples. Previously, we developed an NTA approach to map chemical mixtures in outdoor urban air from passive air samplers (PASs) deployed during summer 2021 across the Island of Montreal, Canada, with twenty-five molecular features confirmed with analytical standards. Additional PAS sampling during different seasons and over different deployment times may yield information on the temporal pattern of the MFs of interest.

Here, we expand the previous study by deploying PASs at the same sapling sites (both residential and public area) in 2024 and 2025, to obtain spatial and temporal trends of chemical features across three different time points. Residues trapped in air samplers were extracted via accelerated solvent extraction and concentrated for LC-Q-TOF-MS analysis. Retrospective data processing and database screening were applied to characterize chemical mixtures of environmental contaminants including pesticides, flame retardants and plasticizers in outdoor urban air. Several of extracted features were successfully identified based on the accurate mass of

parent ion, isotopic patterns, retention time and multiple MS/MS fragment ions with matching abundance ratio. The temporal pattern of previous detected compounds as well as newly identified compounds were compared. This study will provide insight into risk assessment of airborne exposure to chemical mixtures and their effects related to human health.

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3.11.A.T-03 Data-Driven Prioritisation of Transformation Products During Drinking Water Treatment

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Organic micropollutants (OMPs) are widely distributed in the aquatic environment and pose significant difficulties for (waste)water treatment. Advanced oxidation processes (AOPs), such as UV photolysis combined with hydrogen peroxide (H₂O₂), are commonly employed to degrade OMPs in water treatment. However, AOPs often generate numerous unknown transformation products (TPs), some of which may be hazardous. There is therefore an urgent need to understand how TP formation and their potential toxicity are influenced by AOP operating conditions. The main challenge in the characterization of unknown TPs consists of the development of a scalable and comprehensive method to detect and identify TPs, assess their potential (in-vitro) toxicity and link their formation to AOP. This study presents the development of a (bio)analytical platform to link the chemical and hazard space of TPs to the operational conditions of AOPs used in drinking water treatment. The workflow combines chemical analysis using orthogonal liquid chromatography (RP and HILIC), ion mobility separation with high-resolution mass spectrometry (IMS-HRMS), with computational tools to monitor TP formation and removal. Specifically, MS² similarity scores (e.g., Cosine, Tanimoto, MS²DeepScore) were used alongside analytical information (e.g., retention time, m/z, CCS) and K-means clustering to group transformation pathways. The approach was evaluated through controlled degradation experiments in both spiked and unspiked surface water using a pilot-scale AOP reactor. In addition, in vitro bioassays and in silico toxicity prediction tools are integrated to assess biological activity and prioritise compounds of concern. The prioritization workflow proved highly effective in reducing data complexity and focusing on TPs of potential concern, subsequently removing up to 98% of detected features. The prioritisation strategy highlighted distinct patterns in TP formation and persistence under different operational conditions. For example, a flame retardant and three associated TPs were tentatively identified: two TPs were confirmed via MassBankEU, while the third was assigned a probable structure using SIRIUS, CCS and MS² similarity to adjacent features. Although structural elucidation of TPs remains difficult due to the lack of reference MS² spectra and standards, this integrated approach enables a thorough evaluation of both chemical and biological risks associated with AOPs under varying operational conditions.

3.11.A.T-04 Non-target Analysis of Transformation Products in Drinking Water in and around Luxembourg

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Drinking water is a source of human chemical exposure, yet monitoring relies on target analysis, leaving many compounds undetected. Transformation products (TPs) are especially overlooked because they often lack reference standards and may not be well documented. This is evident in Luxembourg, where only eight monitored compounds are pesticide TPs. Non-target screening aims to capture a broader range of contaminants, including TPs. This study applies non-target analysis to identify potentially hazardous TPs and precursors that are not routinely monitored in Luxembourg or neighboring regions.

Tap water samples were collected once a month over three months at 31 locations in Luxembourg and surrounds. 6 brands of bottled water were also analysed. The samples were preconcentrated using solid phase extraction and analysed with reversed phase chromatography coupled with high resolution mass spectrometry. The data analysis was performed using patRoön, while toxicity prediction for endocrine disrupting properties was performed using OPERA.

In total 171 compounds were annotated in the tap and bottled water samples in the first month of sampling. None of the compounds annotated in the bottled water samples are routinely monitored. 110 of the 131

compounds annotated in the tap water samples are also not included in routine monitoring. This shows the importance of non-target analysis to complement target analysis for identification of drinking water contaminants. 50 compounds were pharmaceuticals, indicating the need to monitor this compound class. 12 of the compounds annotated in bottled water and 16 of the compounds annotated in tap water were predicted to have endocrine disrupting properties, indicating that they pose a potential risk to human health. For the tap water samples, 10 of these compounds are not, to our knowledge, routinely monitored. 24 compounds annotated in the bottled and/or tap water were known TPs. Some of these compounds have multiple known precursors, while some of these precursors had substructures that were preserved in the TP. These persistent substructures can be used for grouping efforts in further research to identify other potential sources of these contaminants.

These results can hopefully help inform future drinking water monitoring efforts in Luxembourg as well as other EU countries and drinking water treatment research. It also provides insight into the chemical exposure via drinking water in the Luxembourg greater region.

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3.11.A.T-05 Hidden Volatile Chemicals Indoors: Insights from Non-Target Analysis of European Homes

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People spend most of their time indoors, yet the mixture of airborne chemicals in homes is still poorly characterized. Many airborne compounds are linked to adverse health effects, making the identification of indoor pollutants essential. Non-target analysis provides a broad and unbiased approach to reveal the complex chemical mixtures present in indoor environments. In this study, two complementary strategies were applied to identify chemicals of concern in more than 200 European homes. Silicone foams were used to accumulate semi-volatile organic compounds, while sorbent tubes were deployed to capture volatile compounds. Foams were extracted and analyzed using gas chromatography coupled to high-resolution mass spectrometry. Volatile compounds collected on sorbent tubes were measured by thermal desorption gas chromatography. Statistical analyses were carried out to highlight characteristic indoor chemicals, and information on common uses was combined with a prioritization workflow to identify substances of potential concern. More than 800 chemicals were annotated across both analytical approaches. Most detected features occurred more frequently indoors than outdoors, underlining the significance of indoor exposure. Among the identified semi-volatile compounds, roughly half were natural products and half originated from anthropogenic sources, with many related to additives and personal care products. Plastic additives were found in nearly every home, confirming their widespread presence in indoor air. This supports growing evidence that plastics and their additives contribute not only to aquatic pollution but also to airborne contamination. Volatile organic compound (VOC) analysis revealed hundreds of chemicals in most homes, far exceeding the limited suites of compounds typically included in traditional VOC measurements. Personal care products and their additives emerged as an important class that is often overlooked in routine assessments. Overall, this study demonstrates that indoor environments contain a much broader and more complex mixture of chemicals than previously recognized. Hundreds of natural and anthropogenic compounds were detected using two complementary sampling and GC-HRMS methods, showing that exposure to additives, personal care products, and other indoor pollutants is more extensive than commonly reported.

3.11.B Exploring the Chemical Exposome through Advanced Target and Nontarget Screening: Bridging Occurrence, Transformation Products, and Human Health

3.11.B.T-01 Mapping Human Metabolism in Wastewater – An In-Depth Analysis Using Feature-Based Molecular Networking

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Human excreta and raw wastewater are complementary matrices that capture epidemiological information at the individual and population level. While both provide insights into health status, consumption behavior or

chemical exposure, their chemical complexity differs significantly. Wastewater is far more chemically complex than direct human excretion, as it captures several sources other than human excretion and is affected by dilution, matrix effects and in-sewer processes. Characterizing the overlap and transformation of chemical features from the point of excretion to their detection in wastewater is therefore essential for assessing how accurately wastewater can reflect human excretion.

This pilot study presents the first direct comparison of toilet flush samples and wastewater treatment plant (WWTP) influent using liquid chromatography-high-resolution mass spectrometry. Non-target screening in combination with feature-based molecular networking (FBMN) was applied to map and compare the broad detectable chemical space in both matrices.

Only ~25% of features overlapped between toilet flush and raw wastewater samples, showing that wastewater captures a limited portion of human excretion. FBMN further revealed patterns of feature detectability beyond their excretion, confirming the reliability of well-established WBE markers while highlighting the challenges for using phase II metabolites as potential biomarkers in WBE.

By combining NTS and FBMN, this study presents a powerful approach for mapping human-specific features in wastewater, enabling the identification of reliable biomarkers and evaluation of their stability and detectability across the sewer system. The findings provide new insights into the relationship between human excretion and wastewater influent while providing a new approach to advance WBE, improve exposure assessments, and broaden its applications in public health and environmental monitoring.

3.11.B.T-02 Characterising The Human Perinatal Chemical Exposome with Innovative Suspect and Non-Targeted Screening: Results from The PARC EU Interlaboratory Trial and Real-Case Study

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Early chemical exposures may predispose individuals to various health issues. Perinatal exposure assessment still remain particularly challenging, partly due to limited availability of suitable biological samples for comprehensive analysis. Suspect and non-targeted screening (SS/NTS) approaches open the door to an expanded detectable chemical space, capturing known pollutants and contaminants of emerging concern in a single run. These approaches are thus particularly suited to perinatal exposomic studies.

This work first reports an EU-wide interlaboratory trial combined with real-case profiling conducted by 11 laboratories in the frame of the PARC project. Maternal milk, serum and urine samples were collected from 30 Spanish mothers. Pooled samples of each type were prepared and spiked with 50 predefined QAQC exposure markers at 4 spiking levels. Participating lab applied their own in-house HILIC, RPLC-and/or GC- based HRMS SS/NTS workflows, from sample preparation to data processing.

The methodological assessment of the individual analytical workflows was done by comparing the detection rates and chemical-space coverages of each lab. Results demonstrated a globally good detection even at low spiking levels, and an improved coverage when complementary analytical platforms are combined. A more detailed statistical analysis was conducted by applying univariate and multivariate analysis to rely the captured chemical spaces and the detailed analytical parameters, leading to potential recommendations. A second clustering approach as well as an innovative scoring system for qualitative approaches (S-score) were also applied to describe and discuss the interlab results.

Second, the chemical profiling of all individual samples collected in the study was performed by these different partners. After 3 rounds of data consolidation and curation, more than 1000 markers of exogenous chemical exposure were identified with high confidence levels. These results documented real-life co-exposure to a wide range of compounds, including pharmaceuticals, industrial and food additives, and lifestyle markers. A larger scale real sample analyses campaign is on-going to expand these findings.

Taken together, these results demonstrate the capacity and interest of SS/NTS workflows for characterising

internal chemical exposures in human perinatal cohorts and provide practical guidance on methodological choices that enhance the captured chemical space and comparability across laboratories

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3.11.B.T-03 Comprehensive Urinary Target and Suspect Screening of Endocrine-Disrupting Chemicals in Children and Their Association with Lifestyle Trends

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Children are exposed daily to endocrine-disrupting chemicals (EDCs) through diet, inhalation, or dermal contact, which may pose health risks as they can interfere with estrogenic regulation, affecting child development. Urine, a non-invasive and easily obtainable matrix, is widely used to detect EDCs in exposure studies. Human biomonitoring has become a key tool for assessing exposure in populations.

The objectives of the present study were: (i) to assess exposure to 90 prioritized EDCs using a target screening (TS); (ii) to explore the presence of additional potential EDCs through a suspect screening (SS); and (iii) to examine the relationship between exposure to EDCs and various lifestyle factors (diet, body mass index (BMI), and socioeconomic status) in children.

The samples analyzed were obtained from the INMA cohort and included 350 children aged 8 years from Asturias, Spain. Additionally, 50 of these samples were randomly selected for the SS.

The TS was performed using low-resolution tandem mass spectrometry on an Agilent 6475 LC/TQ system. The SS was carried out using high-resolution mass spectrometry with an Orbitrap Eclipse™ Tribrid™ mass spectrometer, employing a suspected list of over 25,000 compounds.

In the TS, 12 compounds were detected with a frequency greater than 30 %, mostly from the bisphenol and benzophenone families. Non-parametric statistical tests were performed with these compounds to evaluate exposure trends according to lifestyle factors.

The results showed a slight correlation between urinary levels of bisphenol S and fish consumption, which was borderline significant ($p = 0.06$), indicating a trend in which higher consumption was associated with increased concentrations. A similar trend was observed for meat consumption ($p = 0.08$). No association was found with the intake of fruits and vegetables ($p = 0.56$). No significant relationships were observed between the exposure levels of any EDCs and BMI or socioeconomic status.

In the SS, six additional EDCs not included in the target list were tentatively identified with a confidence level of 2a.

These results provide a comprehensive assessment of exposure to EDCs. Furthermore, the limited number of EDCs detected in the SS, beyond those identified in the TS, suggests that the selection of prioritized compounds is appropriate and sufficiently representative. Bisphenol S showed a slight association with the consumption of animal-based foods, suggesting diet as a relevant exposure route.

3.11.B.T-04 Uncovering Environmental Drivers of Steatotic Liver Disease Through Integrated Human and Experimental Evidence

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Metabolic dysfunction-associated steatotic liver disease (MASLD) affects about 25% of the global population and is increasingly recognised as a condition shaped not only by metabolic risk factors but also by environmental exposures. Emerging evidence suggests that endocrine-disrupting chemicals (EDCs) may contribute to MASLD development or progression, however, the specific chemicals involved and their metabolic mechanisms remain insufficiently understood. The aim of this study was to identify EDCs associated with MASLD severity and to elucidate the metabolic pathways through which they may influence liver pathology.

We performed a comprehensive exposome analysis of plasma samples from more than 1500 individuals in the European Steatotic Liver Disease (SLD) registry, integrating targeted EDC quantification with metabolomics and lipidomics using ultra-high-pressure liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Associations between chemical exposures, MASLD stages, and metabolic traits, including insulin resistance were assessed, with additional consideration of sex-specific effects. To support mechanistic interpretation, findings were compared with results from complementary in vivo and in vitro PFAS exposure models.

Perfluoroalkyl substances (PFAS) emerged as the exposure group most consistently associated with severity of liver damage. Metabolome-wide analyses identified clusters enriched in bile acids, lipid species, amino acids, and several gut microbiota-derived metabolites that mediated PFAS–liver trait relationships. In vitro models demonstrated that PFAS directly alter metabolic profiles, particularly impairing mitochondrial metabolism. Importantly, steatotic conditions combined with PFAS exposure produced substantially stronger metabolic disturbances than either PFAS exposure or steatosis alone.

In conclusion, this integrated human–experimental study identifies PFAS as key environmental contributors to severity of liver damage and highlights mitochondrial and gut–liver axis dysfunction as potential underlying mechanisms. These findings emphasise the need to incorporate exposomics into MASLD risk assessment and prevention strategies.

Disclaimer/Disclosure:

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3.11.B.T-05 Inorganic and Organic Exposome Analysis to Investigate Placental Transfer and Pre-eclampsia

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The exposome, the totality of environmental and food-related exposures, is a key driver of human health and disease. Blood and tissue concentrations of chemicals are important internal exposure markers and can impact embryonic development and early life. Trace elements and metals are a crucial yet currently underrepresented part of the exposome. Furthermore, data on placental transfer of many organic chemicals and metals are still scarce, and systematic investigations are typically based on placental perfusion models.

Comprehensive characterization of the early-life exposome requires sensitive multi-analyte assays able to detect diverse chemicals at trace levels in small sample volumes. To overcome limitations of targeted human biomonitoring, we developed a workflow to identify and quantify toxicants and pollutants in low amounts of placenta, maternal blood and cord blood (i.e., 50 mg or 50 µL). Furthermore, we implemented a consistent sample preparation strategy to determine the inorganic and organic components of the human exposome from a single sample aliquot. Sensitive tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) was used for trace analysis and quantification of organic chemicals including plasticizers, perfluoroalkyl substances, personal care products, and food contaminants. Suspect screening using high-resolution mass spectrometry (LC-HRMS) further expanded chemical coverage beyond targeted analytes. Open-source software was used for data preprocessing, feature prioritization, and compound annotation. In addition, multi-element quantification was

carried out by inductively coupled plasma mass spectrometry (ICP-MS), targeting >50 elements, essential micronutrients and toxic metals.

In a pilot study, matched placenta, maternal blood and cord blood samples from participants of a cross-sectional study were analyzed and pre-eclamptic participants were compared with healthy controls. Using our novel approach for the first time, various endogenous compounds and xenobiotics including medical drugs, environmental, food, and lifestyle-related chemicals were annotated, identified and quantified in blood and placenta. The expanded analyte coverage enabled the comprehensive investigation of the metabolome and the organic and inorganic parts of the exposome and allowed assessment of the placental transfer of diverse xenobiotics and metals in real-life human samples.

3.11.P Exploring the Chemical Exposome through Advanced Target and Nontarget Screening: Bridging Occurrence, Transformation Products, and Human Health

3.11.P-Th047 Keep It Clean: Chemical Characterization of Indoor Dust Using Suspect and Non-Targeted Screening

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Indoor dust is a complex and heterogeneous matrix containing a wide variety of organic compounds of different nature and origin. Many of these chemicals pose potential health risks to humans who are exposed to them through inhalation, ingestion and/or dermal uptake. Since population spends about 90% of its time in occupational and residential indoor environments, it is crucial to characterize pollutants present in indoor dust.

The current study proposes a comprehensive characterization of indoor dust collected from homes (n=14), offices (n=22), kindergartens (n=21) and highschools (n=26). Samples were extracted using a QuEChERS method and analyzed with gas chromatography coupled to high-resolution mass spectrometry (GC-QTOF HRMS). Dust samples were processed using a data processing workflow based on an initial suspect screening including more than 1,000 contaminants and comparing mass spectral data towards HRMS-libraries. Complementarily, those samples with a higher pollutant burden were further characterized through non-targeted screening (NTS) to identify and prioritize additional compounds.

More than 250 compounds were identified with the designed workflow at different confidence levels (Schymanski's scale), with 120 compounds being detected in 30% of the samples. Suspect screening revealed up to 61 contaminants detected in a single sample including pesticides, flame retardants and other industrial compounds. NTS aided to characterize 49-88 contaminants per sample, including other emerging contaminants (ECs) such as antioxidants (n=11) and their transformation products (n=8), non-phthalate plasticizers and plastic additives (n=10), UV filters (n=6) or photoinitiators (n=2). Some of the identified compounds, such as the transformation product of the antioxidant Irgafos 168, tris(2,4-di-tert-butylphenyl) phosphate (AO168?O), or the plastic additive 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, are considered pollutants of emerging concern and stood out in the analyzed samples.

In general, the developed strategy provided a more complete understanding of the presence of emerging compounds in indoor environments. Understanding indoor EC sources and their distribution is critical for assessing human exposure, especially for vulnerable who have greater dust contact.

Disclaimer/Disclosure:

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3.11.P-Th048 Non-target Screening and Toxicity-Based Prioritization of Industrial Chemicals in Coastal Sediments

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Coastal industrial regions such as Busan Bay are exposed to diverse mixtures of organic chemicals from shipbuilding, port logistics, and land-based industries. Sediments act as long-term sinks for both regulated and

emerging contaminants, making comprehensive chemical characterization essential. To achieve this, gas chromatography time-of-flight mass spectrometry (GC-TOF/MS) based non-target screening (NTS) was applied to surface sediments (n = 30) collected from shipbuilding areas, port terminals, and mixed industrial regions. Standard NTS workflows, including alignment, filtering, and spectral matching, were used to curate environmentally relevant chemicals classified into organosilicons, benzotriazole ultraviolet (UV) stabilizers, phthalates, polycyclic aromatic hydrocarbons (PAHs), heterocycles, organochlorides, and organotins.

Multivariate analysis of log-transformed intensities showed clear spatial differences. Principal component analysis (PCA) separated sediments into three groups representing shipbuilding, port logistics, and mixed regions, while hierarchical clustering (HCA) grouped chemicals according to these source influences. Organosilicons, benzotriazole UV stabilizers, and phthalates were enriched in shoreline industrial and port-associated areas, whereas PAHs, heterocycles, organochlorides, and organotins were characteristic of shipyard and industrial-complex sectors.

Relative toxic units (rTU), calculated by normalizing intensities with chronic toxicity values, showed that several subset of chemicals dominated potential toxicity. Organosilicons displayed the highest rTU values and were the strongest toxicity drivers. Organotins were the second major contributors, consistent with antifouling and ship-maintenance inputs. High-molecular-weight phthalates contributed more than low-molecular-weight plasticizers. Benzotriazole UV stabilizers showed moderate contributions, while PAHs and organochlorides contributed at secondary levels, reflecting both ongoing discharges and legacy pollution.

This study shows that combining NTS, multivariate clustering, and rTU evaluation effectively identifies characteristic chemical fingerprints and high-risk contaminants in coastal sediments. The dominant influence of organosilicons, organotins, and high-molecular-weight phthalates highlights the need for monitoring strategies addressing both emerging and persistent pollutants in industrialized marine environments.

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3.11.P-Th049 Necrological pollution of the environment through human burial site leachate

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The environmental impacts of human burial practices remain understudied despite their potential to introduce a wide range of organic micropollutants (OMPs) into surrounding ecosystems. As populations grow and life expectancy increases, the number of individuals undergoing intensive pharmaceutical treatment at the end of life is expected to rise, raising concerns about the chemicals that may enter the environment through burial. Furthermore, traditional burial materials (including wooden coffins, varnishes, flame retardants, plasticizers, and embalming fluids such as formaldehyde) introduce additional contaminant sources that may leach into soil and water over time. This study investigates the extent of necrological pollution at the Berthåga municipal cemetery in Uppsala, Sweden, as a case-study where preliminary observations indicated abnormal toxicity and the presence of endocrine-active substances in a stormwater pond connected to a creek flowing through the burial grounds.

To evaluate the potential leaching of OMPs from the cemetery, water and sediment samples were collected at multiple points across the burial site and along upstream and downstream sections of the receiving Fyris River. Samples were subjected to solid-liquid extraction (for sediments) followed by multi-layer solid-phase extraction to cover a broad range of chemical properties and were subsequently analyzed using liquid chromatography coupled to high-resolution mass spectrometry. A suspect screening approach against pharmaceuticals, hormones, industrial chemicals, and flame retardants was used to identify potential contaminants.

The results revealed numerous OMPs in both water and sediment matrices, with two groups (flame retardants and industrial derivatives) showing particularly high occurrence within the burial site. Pharmaceuticals were also frequently detected, indicating that biological fluids from decomposing remains are reaching adjacent water bodies. Collectively, the data demonstrates that human burial sites can act as diffuse but significant sources of environmental contamination. These findings highlight the need to critically reassess burial-related infrastructure and management strategies to safeguard long-term environmental and public health.

3.11.P-Th050 Expanding the Scope of High-Throughput Effect-Directed Analysis with Orthogonal Separation Modes and Ion Mobility to Identify Endocrine-Disrupting Chemicals in Outdoor Stormwater Ponds

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High-throughput effect-directed analysis (HT-EDA) has been a powerful tool for prioritizing and identifying unmonitored endocrine-disrupting compounds in complex environmental mixtures. Its effectiveness can be greatly enhanced when coupled with multidimensional chromatography and ion mobility separation - high-resolution mass spectrometry (IMS-HRMS). This approach allows to expand chemical coverage, improve separation of coeluting and isomeric compounds, and increase confidence in compound annotation. The present study applies this expanded HT-EDA strategy to identify endocrine-active transthyretin (TTR)-binding chemicals in stormwater ponds, which are hotspots for urban-derived micropollutants. Water from outdoor stormwater ponds around Uppsala (Sweden) was collected and concentrated through solid-phase extraction (SPE). The stormwater extracts were screened on their ability to compete with thyroid hormone (TH) for binding to TTR, the TH distributor protein in plasma and cerebrospinal fluids. The extracts with higher binding affinity to TTR were selected for parallel fractionation into 96-well plates using two orthogonal separation modes: hydrophilic interaction chromatography (HILIC) and reversed-phase liquid chromatography (RP). The fractions were screened on a fluorescent competitive TTR-binding assay, and the chemical analysis of the same extracts was performed using trapped ion mobility-quadrupole time-of-flight MS. Suspect and nontarget screening was performed, and compound annotation was focused on features within fractions exhibiting high TTR-binding activity. The workflow revealed several candidates whose TTR-binding activities can be confirmed from literature, or can be prioritized for future testing. The additional dimensions in this strategy helped in addressing critical bottlenecks in EDA. Incorporating HILIC as a separation method improves the chemical coverage of EDA, allowing improved fractionation of complex mixtures containing polar or ionized compounds, which are not retained in conventional reversed-phase chromatography. In the nontarget screening of the active fractions, incorporating ion mobility as a dimension helped to distinguish analytes from coeluting matrix interferences, while acting as an additional line of evidence that increases the confidence level of the annotated features.

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3.11.P-Th051 Determination of Estrogens in Drinking and Surface Water at low ng/L levels by direct injection using ACQUITY™ Premier LC System coupled to the Xevo™ TQ Absolute Mass Spectrometer **Sara Beverley, Ofure Idialu and Peter Hancock, Waters Corporation, United Kingdom**

Estrogens such as 17 α -ethinylestradiol (EE2), 17 α -estradiol, 17 β -estradiol (E2), estrone (E1), and estriol (E3) are endocrine-disrupting compounds of significant environmental concern, three (EE2, E2, and E1) with extremely low Environmental Quality Standards (EQS) proposed under the EU Water Framework Directive (WFD). Achieving these ultra-trace levels (0.017–0.36 ng/L) has traditionally required labor-intensive sample preparation steps, including derivatization and large-volume extraction (up to 1 L).

This study presents a highly sensitive LC-MS/MS method using the ACQUITY™ Premier System coupled to the Xevo™ TQ Absolute Mass Spectrometer for direct injection analysis of estrogens in drinking and surface water. Method performance was successfully evaluated using matrix-matched calibration curves, achieving excellent sensitivity (1 ng/L) and selectivity for all five target estrogens.

Baseline separation of 17 α -E2 and 17 β -E2 was obtained using a 150 mm column, with robust retention, resolution, and peak shape for all analytes within an 8-minute run time. Response was linear over a range of 1 - 500 ng/L, with ion ratios and RSD values (<10%) meeting required tolerances, demonstrating repeatability and accuracy at lowest ng/L levels. Matrix effects were evaluated (16–29%) and found acceptable for both drinking and surface water.

Increased sensitivity enables the use of reduced sample volumes (one-tenth of the previously required 1 L), significantly decreasing analysis time and chemical waste. These results confirm that this streamlined workflow supports compliance with revised EU WFD requirements while improving laboratory efficiency and sustainability.

3.11.P-Th052 Bridging Chemical Analysis and Bioassays in Breast Milk to Assess Early-life Exposure

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Humans are daily exposed to a wide variety of chemicals, many of which are bioactive and may contribute to adverse health outcomes. Within the "One Health" framework, this study aims to develop a holistic extraction methodology for xenobiotics with diverse physicochemical properties in breast milk and to integrate it into an effect-based approach to better assess potential toxicological impacts relevant to infant development. Several extraction protocols were evaluated, using as a starting point an existing method for human plasma combining polydimethylsiloxane (PDMS) disks extraction with solid-phase extraction (SPE). Given the high lipid and protein content of breast milk, additional clean-up steps were evaluated: lipid removal using Captiva Enhanced Matrix Removal (EMR) cartridges in combination to PDMS, and a low-temperature protein precipitation (PP) with acetonitrile (AcN) and hexane. Polar and semi-polar contaminants were analyzed via liquid chromatography-high resolution mass spectrometry (LC-HRMS), while hydrophobic compounds were assessed with gas chromatography-high resolution mass spectrometry (GC-HRMS). A battery of bioassays was applied to assess developmental neurotoxicity, mitochondrial toxicity, oxidative stress response, immunosuppression and androgen receptor antagonism. Results underscored the challenges of integrating chemical analysis and bioassays into a single workflow. PDMS extraction improved the recovery of hydrophobic compounds compared to extraction with hexane, while EMR clean-up showed to be a relevant step, with around 50% of the recovered compounds falling within the acceptable recovery range (60%-140%). Largest losses were observed for hydrophobic compounds ($\log K_{ow} > 3$), although EMR clean-up slightly improved their extraction efficiency. Effect recoveries for developmental neurotoxicity aligned with the chemical recoveries, with PP using AcN and hexane performing the worst. In contrast, the combination of PDMS with PP (AcN) and EMR-SPE preserved significant fractions of bioactive chemicals, showing mean effect recoveries of around 55% and 25%, respectively. These findings suggest that hydrophilic compounds may be major contributors to the observed activity. Further experiments will include additional toxicological endpoints, like mitochondrial toxicity, oxidative stress response, immunosuppression and androgen receptor suppression, to improve toxicological coverage across a wider range of disease-related mechanisms.

3.11.P-Th053 PREVALENCE OF URINARY TRICLOSAN AMONG ADVANCED LUNG CANCER PATIENTS IN VOJVODINA

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Environmental pollution as a major risk factor alongside smoking came into focus of lung cancer studies. Urinary triclosan concentrations were significantly associated with breast cancer risk, although currently there is no direct evidence in human data establishing a link between triclosan exposure and the lung cancer development. In this research the prevalence of urinary triclosan among newly diagnosed advanced stage lung cancer patients of both genders was determined.

A total of 190 patients (44.7% female) with advanced stage (III/IV) lung cancer diagnosed at the Institute for Pulmonary Diseases of Vojvodina, Serbia, were enrolled: 61 patients with small-cell lung cancer (45.9% female), 67 patients with adenocarcinoma (41.8% female) and 62 patients with squamous cell cancer (48.4% female). Triclosan was analyzed using gas chromatography-mass spectrometry in the first morning urine sample within 24h of hospitalization.

In 29.5% (56/190) urine samples triclosan was measured above limit of quantification with average concentration 0.161 ± 0.141 $\mu\text{g/L}$ or 0.535 ± 1.070 $\mu\text{g/g}$ creatinine. There were no differences in frequency of quantification between genders (27.6% male vs. 31.8% female). However, there was 3.33 times (95%CI 1.48-7.50, $p=0.037$) higher chance of urinary triclosan quantification in adenocarcinoma patients (41.8%) in comparison to squamous cells cancer ones (17.7%) with no differences when compared to patients with small-

cell lung cancer (27.9%). This trend was more pronounced among females with adenocarcinoma (51.8%) vs. squamous cell cancer (20%) with 4.31 higher chance (95%CI 1.37-13.89, $p=0.0145$) of quantifying triclosan. The odds for triclosan presence among males with adenocarcinoma (35%) vs. squamous cell cancer (15.6%) was 2.91 times higher (95%CI 0.92-9.22, $p=0.07$). Finally, triclosan was 3.23 times more often quantified (95%CI 1.03-10.11, $p=0.044$) among females with adenocarcinoma (51.8%) vs. small-cell lung cancer counterparts (25%).

Almost one-thirds of patients enrolled in this study had triclosan in their urine sample with highest frequency of quantification among patients with adenocarcinoma. Further studies are urgent in order to determine its influence on survival rate among adenocarcinoma patients non-occupationally exposed to triclosan and detecting the probable sources of exposure.

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3.11.P-Th054 Prioritisation framework to identify relevant substances for assessing the impact of environmental contaminants on human exposure

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Humans are exposed to organic compounds from various sources, including indoor air, dust, drinking water, and garden soil - areas less systematically assessed than food. Chemical analyses of these matrices can provide valuable insights into which environmental sources contribute to the presence of contaminants in humans. Since monitoring studies are both resource- and time-intensive, optimising monitoring strategies is essential to ensure efforts focus on the most relevant compound groups, environmental matrices, and endpoints. Within the Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC) a multicriteria prioritisation process, inspired by the NORMAN prioritisation scheme based on action categories, was proposed to identify relevant (groups of) substances to monitor in indoor air, drinking water, indoor dust and soil, aiming to assess the impact of environmental contaminants on human exposure.

An initial list of approximately 90,000 substances was created using a comprehensive literature review, the NORMAN Suspect List Exchange and the EU LCI list as sources. Ranking criteria were developed to prioritise substances based on use, toxicity, hazardous classification, physical properties and occurrence data where available. Matrix-specific decision trees were developed for the four matrices. According to the decision trees the initial list was enriched with further data to create a master list.

As a result of the application of the decision tree, approximately 500 substances for soil, 4,800 for indoor dust, 4,000 for indoor air, and 1,700 for drinking water were prioritised and classified into two tiers: (1) targeted analysis candidates and (2) suspect/non-target screening (SS/NTS) candidates. Suspect screening can provide an initial overview of potentially present substances, enabling more focused follow-up via target analysis.

The decision tree approach was successful to prioritise relevant lists of compounds using a systematic, transparent and reproducible ranking approach. The findings from this study, together with an additional expert judgment step, will serve as recommendations for monitoring contaminant classes that are currently insufficiently investigated in the environment. This will deepen the understanding of the impact of environmental contaminants on human health.

Disclaimer/Disclosure:

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3.11.P-Th055 Positive Control Substances for Experiments Described in the EFSA Guidance on the Impact of Water Treatment Processes

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The European Food Safety Authority (EFSA) guidance document on the impact of water treatment processes describes a series of experiments for the generation and identification of transformation products. These processes include chlorination, chloramination, oxidation, ozonolysis, and ultraviolet light (UV) treatment.

To confirm that the test system was correctly prepared and has the potential to form transformation products, a positive control is needed. The positive control can be achieved using an indirect method, for example measurement of the reagent concentration using a colorimetric method with N,N-diethyl-p-phenylenediamine (DPD) or indophenol.

Alternatively, a direct method may be used, i.e. a compound that degrades under the reaction conditions. In this case, the selected compound(s) should have the following characteristics:

- Degrade reliably and reproducibly
- Degrade under all of the conditions to be tested
- Readily available commercially
- Relatively inexpensive
- Easy and safe to handle

Additional points may also be considered. For example, a compound that produces transformation products covering a wide range of polarities may also be used as a quality control for analytical work. Here, we present results of experiments to test various compounds for these properties.

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.

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.

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 using a non-target screening workflow, while quantitative analysis was performed by HRAM or multiple reaction monitoring (MRM) on a triple quadrupole mass spectrometer.

3.11.P-Th056 Challenges in the Annotation of Environmental Contaminants with Nontarget Screening - Quality and Information of Tandem Mass Spectra

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Advances in high-resolution mass spectrometry (HRMS) have driven a shift toward broad screening methods in environmental analysis. Central to these methods are tandem mass spectra (MS2), used for feature annotation, (sub)structure prediction, and toxicity estimation, however the MS2 data quality greatly affects this. A machine learning model was recently developed to assess MS2 quality using mathematical features. While it aligns with expert-based assessments, discrepancies remain when compared to identification scores from such as SIRIUS. To clarify these differences, this study investigates diagnostic patterns in MS2 spectra and biases across chemical space.

Machine learning models are applied to predict the probability of correct annotation and to examine how chemical-space coverage, MS2 quality, and retrieval performance relate, using experimental and library spectra from various instruments and sample types. In total, 145 mathematical MS2 descriptors were computed to characterize spectrum quality, i.e. the likelihood of successful annotation. The best model, based on 18 optimized descriptors, achieved 73% accuracy and 86% precision. Feature-importance analysis revealed clear quality-related trends, with negatively contributing effects often linked to noise or insufficient or excessive fragmentation.

Applying the model trained on neat reference standards to experimental datasets from diverse instruments and sample types revealed shifts in descriptor distributions, indicating that parts of the data fall outside the model's applicability domain. These shifts suggest that, for certain data types, MS2 quality can negatively affect

annotation outcomes. Ongoing work will investigate the causes of inconsistencies between experimental and library spectra and improve understanding of instrument- and matrix-related variability.

In conclusion, the model can prioritize spectra more likely to yield correct annotations. Understanding which spectral characteristics provide diagnostic value may improve molecule retrieval, increase library match rates, and support better-curated libraries. Filtering low-information spectra from libraries and experimental datasets can further increase annotation success, and focusing on information-rich spectra improves efficiency and reduces costs. Downstream applications such as toxicity prediction, structure annotation, and related predictive models will likewise benefit from the removal of low-informative spectra.

3.11.P-Th057 Retention index alignment algorithm for the alignment of data from different NTS studies without requiring shared standards

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Non-target screening (NTS) through liquid chromatography coupled to high resolution mass spectrometry (LC-MS) is valuable to environmental monitoring because of the ability to detect a broad range of (un)known organic micropollutants (OMP's). Laboratories are frequently acquiring a lot of NTS monitoring-data of drinking-, ground- or surface-water. Comparison of their NTS-datasets allows more comprehensive analysis of OMP's occurrence, aids prioritization of unknown features, and can help identification of unknown features. The alignment of retention time is one of the key aspects to compare datasets. However, differences between batches or between laboratories, such as analytical methods and equipment, can make it impossible to compare their measured retention times, especially when their standards have little or no overlap. Recently, retention indices (RI) for LC were introduced, enabling comparison of RI values via a set of standards that is injected every batch. However, this constitutes a large and expensive challenge to jointly organize, and would exclude historic or future sample batches that do not contain the same standards.

We present a retention alignment algorithm based on predicted RI for the alignment of NTS-data across time and space without the need for overlapping standards.

To enable alignment without overlapping standards, the algorithm operates by applying a RI prediction model on a number of known analytes measured in the same analysis batch. Their retention time to RI relationships are used to build a model that maps a measured retention time to a probability distribution in the generalized RI space. The features' RI distributions are subsequently combined with their respective m/z values to align them across time and space. We explored advisable numbers of input standards, several strategies to build the retention time to RI model and several strategies to perform alignment using the RI and m/z values. The algorithm is evaluated with data from 4 laboratories across the Netherlands. Altogether, comparing of data from different methods has the potential to advance routine NTS monitoring programs.

3.11.P-Th058 A quantitative structure-response relationship model to estimate concentrations of plasticizer metabolites identified in urine using LC-HRMS without reference standards

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Plasticizers are characterized as endocrine disruptors, and human exposure to these chemicals can adversely affect the reproductive system. Non-targeted analysis (NTA) using liquid chromatography – high resolution mass spectrometry (LC-HRMS) is a discovery-based approach for screening and identifying hundreds of compounds and metabolites in human samples without a priori knowledge of the chemical species present in the sample. However, while NTA methods need to be standardized in order to provide reliable and reproducible results on identification of unknown chemicals/metabolites, it is crucial to provide quantitative concentrations for these compounds identified in NTA to support exposure assessment. Therefore, there is an urgent need to develop quantitative NTA (qNTA) methods. Isotopically labeled internal standards (IS) are routinely used in targeted analyses to correct for the effects of sample matrix and ESI conditions but have not yet been considered for qNTA model development. In this study, we describe a quantitative NTA model using LC-electrospray ionization (ESI)-HRMS that combines quantitative structure-response relationship (QSRR), LC retention behavior, and IS for signal normalization. This relationship can be used to estimate the signal response factors of chemicals and approximate the concentrations of plasticizer metabolites without reference standards. The model consists of 12 variables, (retention time and 11 molecular descriptors), and was able to estimate response factors for eleven test chemicals with an error less than 30%. The study demonstrated that the model can be updated with routine (e.g. daily) calibration curves and estimate concentrations of unknown metabolites in urine with an error less than 50% for most compounds. The inclusion of IS in NTA workflows can reduce the variation in

results introduced during sample preparation, injection and ionization, and data analysis, thus broadening the application of models between different types of instruments (e.g. with significant response factor (RF) difference). Our approach enables a fast and simple estimation of the concentration of unknown metabolites of phthalates and their alternative chemicals without the need for respective reference standards. More importantly, the method is applicable to routine NTA studies and can be adapted to different LC-MS systems (Agilent QTOF and Thermo Orbitrap) by simply updating the model with daily calibration data without having a significant impact on its prediction accuracy.

3.11.P-Th059 Modernizing the Analysis of Flame Retardants in Environmental Matrices: Eliminating the Use of Helium Carrier Gas With Gas Chromatography Atmospheric Pressure Chemical Ionization (GC-APCI)

David Gould¹, Douglas Michael Stevens², Aron Dekkers¹ and Peter Hancock¹, (1)Waters Corporation, United Kingdom, (2)Waters Corporation, Germany

Polybrominated diphenyl ethers (PBDEs) are persistent flame retardants that undergo long range environmental transport through volatilization and deposition cycles in a process known as the grasshopper effect. This mechanism moves PBDEs from warmer to colder regions, resulting in their accumulation in air, soil, water, and biota even in areas where they were never manufactured. Their persistence and global distribution led to inclusion in the Stockholm Convention, which mandates monitoring of PBDEs and other persistent organic pollutants (POPs) across 186 countries.

Historically, PBDE analysis relied on capillary gas chromatography (GC) coupled with high resolution magnetic sector mass spectrometry (HRMS) using electron ionization (EI) for high sensitivity and specificity. However, EI GC-HRMS use is declining due to high cost, maintenance demands, and expertise requirements. This study evaluates GC atmospheric pressure chemical ionization (GC-APCI) combined with tandem quadrupole (TQ) mass spectrometry (MS/MS) as an alternative. The GC-APCI TQ system demonstrated improved speed and robustness while maintaining equal or better sensitivity and specificity compared to EI GC-HRMS. Additionally, the instrument's use of a single nitrogen supply for carrier, reagent, and collision gases supports sustainability goals.

The optimized method achieved a 27-minute runtime, with BDE 209 eluting at 22 minutes with symmetric peak shape ($a/b = 0.993$) and 10.3 s width. Calibration curves for 25 analytes were linear across three orders of magnitude (average $r^2 = 0.999885$), with parts per trillion (ppt) limits of detection. These results indicate that GC-APCI TQ MS/MS is a fit for purpose, sustainable alternative for PBDE monitoring, aligning with global regulatory requirements and advancing analytical capabilities for persistent organic pollutants.

Disclaimer/Disclosure:

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3.11.P-Th060 Detection of Surfactants using Liquid Chromatography Coupled with High Resolution Mass Spectrometry

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At the Bavarian Environment Agency each year approximately 250 to 300 incidences of local environmental accidents are elucidated analytically. In case a surface water contamination shows foam building, one focal point of analytical investigation are surfactants.

A liquid chromatograph with a RP-C18 column coupled with a high resolution mass spectrometer ($R = 120,000$) is used to analyse surface water samples for the presence of surfactants.

First step is to check the measurability of reference substances with the instrumental method and to establish a database for surfactants. With the instrumental method 76 substances within the following 7 different surfactant groups can be determined: alkyl ether sulfates, alkyl sulfates, linear alkylbenzene sulfonates, secondary alkyl sulfonates, alkyl ethoxylates, alkyl benzyl dimethylammonium compounds and alkyl trimethylammonium compounds

Due to the overall use of surfactants in different products of daily life, rather high laboratory blank values occur. Therefore, second step is to minimize blank values but also to find a strategy how to work with unavoidable blank values. A cause effect diagram is the starting point to find trigger points which cause blank values. Finally, it is aimed to express semi-quantitative results for the different surfactant groups.

3.12.A Fate and Toxicity of Metals: Recent Scientific Advancements and Their Application in Environmental Regulations

3.12.A.T-01 Source Contributions to Copper in the Danube River: Integrating Emission Apportionment with Fate Modelling

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Copper (Cu) enters European rivers from multiple sources, including agriculture, road traffic, wastewater, and natural background. Internal recycling from river sediments may also contribute to Cu levels in the water column, but the magnitude of this contribution remains uncertain. This study integrates recent country-level source apportionment for Cu loads with a river fate and transport model to assess how spatially distributed loads translate into water column concentrations in the Danube River.

Cu emissions from twelve source categories were quantified using activity rates from the Danube Hazardous Substances Model (DHSM) and emission factors developed in recent European assessments. The model predicts time-variable water column Cu while accounting for net settling of particulate Cu. To evaluate potential release of historically deposited Cu, a simplified box model was developed that incorporates settling, sediment-water diffusive exchange, resuspension, and burial. A tracer was implemented to track historical Cu released from sediment.

Spatially resolved Cu loads were applied in both models and compared with monitoring data from the Danube River Basin for 2015 to 2021. Although some deviations were observed, both models reproduced the overall magnitude of Cu concentrations along the river. The DHSM was then used to assess contributions from individual sources by running scenarios in which each source category was activated independently. Results show that road-runoff inputs, soils and natural background, and livestock manure are major contributors, with the relative importance of these sources varying between the upper and lower Danube. Preliminary results from the simplified box model indicate that the tracer approach can quantify the influence of legacy Cu released from sediments, though further refinement is needed.

This work demonstrates that country-level source apportionment can be integrated into river-scale fate and transport models to interpret observed Cu concentrations and support evaluation of sediment contributions. The combined framework provides a useful tool for managing Cu and other constituents in large river systems.

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3.12.A.T-02 Accumulation, subcellular distribution and toxicity of indium in the green alga *Chlamydomonas reinhardtii*

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Indium (In), a trivalent hard-acid metal is increasingly used in electronic industries, yet its bioavailability and toxicity to organisms remain poorly documented. The aim of this study was to provide additional knowledge on its impact on microalgae as well as on the detoxification mechanisms developed by algae. The model green alga *Chlamydomonas reinhardtii* were thus exposed for 96 h to indium concentrations ranging from 8×10^{-9} to 4×10^{-7} M and examined for their growth and subcellular parameters. Intracellular indium concentration was also assessed following cellular washing with ethylenediaminetetraacetic acid and its subcellular fractionation was performed using differential ultracentrifugation. Indium speciation in the cytosol was further characterized using size-exclusion chromatography coupled with inductively coupled plasma mass spectrometry (SEC-ICP-MS). Furthermore, indium speciation in exposure media was calculated using thermodynamic modeling with Visual MINTEQ 4.0.

Chlamydomonas reinhardtii tolerated indium concentrations up to $36.5 \mu\text{M}$ without significant effects on growth, chlorophyll a fluorescence, cellular size, and granularity and oxidative stress. Internalized indium was observed to plateau starting at $4.2 \mu\text{M}$, while surface-adsorbed indium continued to increase with exposure. This phenomenon was explained by the extensive hydroxide precipitation of indium, limiting its bioavailability.

Internalized indium was mainly localized in granular and organelle fractions, with only minor association with heat-stable or denatured proteins, suggesting detoxification via sequestration in phosphate-rich compartments rather than thiol-based binding. Analysis of cytosol fraction with SEC-ICP-MS revealed that indium was predominantly bound to >470 kDa biomolecules and to low-molecular-weight ligands <10 kDa. Overall, indium displayed low bioavailability and limited toxicity to microalgae under near-neutral pH conditions. Its intracellular behavior closely resembles that of other hard trivalent metals, with chemical speciation, precipitation, and compartmentalization governing both accumulation and biological impact.

3.12.A.T-03 Bioaccumulation of radium-226 in the freshwater snail *Lymnaea stagnalis*: water quality effects and internal distribution

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Radium-226 (²²⁶Ra) is a naturally occurring radionuclide that may be released into aquatic ecosystems from mining operations. It may be of concern to aquatic ecosystems, because of its long half-life (1600 years), its potential for bioaccumulation and its radiotoxicity. Yet, there is currently very few ²²⁶Ra ecotoxicological data for aquatic organisms, creating uncertainties on the ecological risk assessment (ERA) of this radioelement in fresh waters. Notably, there is very a lack of knowledge on the environmental factors influencing ²²⁶Ra bioavailability to aquatic organisms. Our first objective was to evaluate how water quality may influence ²²⁶Ra bioavailability (and thus toxicity) in a model invertebrate, using the Biotic Ligand Model (BLM) framework. In the lab, we conducted aqueous ²²⁶Ra bioaccumulation experiments with the great pond snail *Lymnaea stagnalis* at varying water quality (pH, DOC, hardness and Ca). Data seem to agree very well with BLM predictions, with water hardness being by far the most important bioavailability modifying factor, as expecting from the strong analogy between ²²⁶Ra and Ca. The data suggests that, at the very least, a hardness-based water quality guideline should be considered for ERA. A second objective was to investigate the internal fate of ²²⁶Ra in juvenile *L. stagnalis* (namely its tissue and subcellular distributions), to gain insights into potential biological targets and detoxification mechanisms. We found that ²²⁶Ra was most concentrated in the Ca carbonate shell, where it is essentially detoxified. The various soft tissues (mantle, hepatopancreas, etc.) had similar ²²⁶Ra concentrations, suggesting that no particular organ can be considered a target of ²²⁶Ra toxicity. Overall, total soft tissue accumulation ²²⁶Ra was moderate compared to other trace metals tested for *L. stagnalis* under similar exposure conditions, possibly because of a tight Ca-like homeostatic control and incorporation of internalized ²²⁶Ra into the growing shell. A longer ²²⁶Ra exposure may eventually lead to relatively great ²²⁶Ra contents in the shell, but the radiological consequences of such external accumulation would likely be limited compared to a long-term ²²⁶Ra build-up within the endoskeleton of vertebrates. Subcellular distribution data are currently being collected to explore the intracellular handling of this radionuclide.

3.12.A.T-04 Mercury and Methylmercury Bioaccumulation in Caged Gammarids Across Lotic Systems of the Rhine-Meuse River Basin (France)

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The distribution and toxicity of mercury (Hg) in lotic ecosystems are largely determined by its chemical speciation. In the upper anoxic layers of sediments, inorganic mercury can be converted to methylmercury (MeHg), its most toxic form, which readily bioaccumulates and biomagnifies through food webs. In France, caged gammarids (*Gammarus fossarum*) are used for routine chemical monitoring, including HgT concentrations. HgT concentrations are being used as a proxy of MeHg concentration in gammarids but this relationship remains poorly documented in benthic invertebrates, raising uncertainties about the reliability of this proxy.

To address this issue, we deployed caged gammarids at 27 sites in the Rhine-Meuse watershed to assess the MeHg bioaccumulation of caged gammarids under environmental conditions, evaluate the variability of the methylmercury percentage (%MeHg) and determine the spatial variation of MeHg across sites in caged gammarids. Sites were selected to cover a wide range of HgT concentrations (<0.05 – 300 ng.g⁻¹ dw). HgT concentrations were measured by direct mercury analyzer (DMA-80) and MeHg concentrations by gas chromatography coupled to inductively coupled plasma mass spectrometry (GC-ICPMS).

The HgT and MeHg concentrations averaged respectively 39.9 ± 8.6 and 26.5 ± 4.8 ng.g⁻¹ (mean $\pm$ sd), which was in the lower range but consistent with the literature and available data. Still, both analytical methods allowed site differentiation. The methylmercury percentage varied from 49 to 93%, which suggests that

gammarids may accumulate MeHg and HgT differently depending on environmental conditions. Thus, the currently used metric HgT in caged gammarids may not be a reliable proxy of Hg contamination in streams.

To better assess site-specific effects on bioaccumulation dynamics of MeHg, we calculated the normalized difference (ND) between individuals prior to exposure (control) and those after exposure. Four significantly distinct site groups were then identified by hierarchical clustering. The spatial variability of ND MeHg was consistent with the historical metal contamination in the Rhine-Meuse river basin, mainly located within the Rhine watershed. Overall, MeHg measurement in caged gammarids showed good signs of usability as a proxy for Hg contamination in lotic systems but further investigation is needed, notably a better understanding of the MeHg toxicokinetics in gammarids.

Disclaimer/Disclosure:

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3.12.A.T-05 Evaluating the impacts of nickel on Arctic freshwater biota, from Crustaceans to Fish

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Nickel (Ni) is an economically important metal used primarily in the manufacture of stainless steel and other metal alloys, with global Ni production exceeding 2.5M tonnes annually. Substantial Ni mining and processing occurs in Arctic and near-Arctic regions, with the number and scale of operations growing. Such extensive natural resource exploitation leads to release of contaminants into the environment, necessitating the evaluation of the risk these contaminants pose to biota. Biotically, the low metabolic rate and large size in Arctic animals may slow toxicant uptake, but low energy usage may limit detoxification potential. Additionally, slow growth rates mean Arctic species spend more time in sensitive larval and/or juvenile stages. Limited polar toxicity data indicates polar species have large variability in toxicant susceptibility. Therefor, we exposed juvenile Arctic char to a range of environmentally relevant Ni concentrations, ranging from 20 – 2400 µg/L for sixty days to investigate the consequences of chronic Ni exposure and the physiologic mechanisms of toxic action. This work evaluated impacts on growth and survival, respiratory toxicity via gill histology and metabolic oxygen consumption, and ionoregulatory perturbation via tissue specific ion analysis and Ni accumulation. These experiments found 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. Unfortunately, the currently available results show Ni contamination poses a risk to various Arctic relevant species at concentrations well within those observed downstream of Arctic Ni mining operations. These results suggest that extrapolation of existing temperate risk assessments to Arctic systems may inadequately protect unique Arctic biota due to both high bioavailability and species sensitivity. Further research is needed to better evaluate the risks of in the Arctic.

3.12.B Fate and Toxicity of Metals: Recent Scientific Advancements and Their Application in Environmental Regulations

3.12.B.T-01 Chronic Toxicity of Metal-Organic Mixtures to *Daphnia magna*: To What Extent Can Concentration Addition and Independent Action Predict Effects?

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Freshwater systems are often exposed to a variety of different chemicals with unknown mixture effects. The mixture effects of metals and organic micropollutants (OMPs) are currently understudied. Most mixture toxicity studies focus on compounds that belong to the same or a similar chemical group. The toxicity of chemical mixtures can be predicted with two reference models, Concentration Addition (CA) and Independent Action (IA). The CA model is widely established in chemical risk assessment and assumes that all compounds in a mixture have the same Mode of Action (MoA). Mixtures with different MoA, such as metals and OMPs, are in theory better described with the IA model. In this study, we evaluated which reference model, CA or IA, describes the toxicity of metal-OMP mixtures best. Chronic *Daphnia magna* reproduction tests were performed

to test the effects of binary mixtures of one metal (Cd, Cu, Ni, or Zn) and one OMP (fenazaquin, fluoranthene, methomyl, or triclosan). To ensure the environmental relevance of the tested mixtures, combinations were selected from a previously published prioritization study that used freshwater monitoring data. The Mixture Interaction Factor (MIF) was used to quantify deviations between observed and predicted effects by dividing the observed effect concentration (EC) by the predicted EC, both being expressed as Sum of Toxic Units (?TU). For metal-OMP mixtures, CA tends to overestimate toxicity (median MIF: 1.42), whereas IA tends to underestimate mixture toxicity (median MIF: 0.84). However, IA predicted the toxicity of binary mixtures more accurately than CA. Especially when test concentrations were expressed as ?TU relative to the EC50 (median MIF, IA: 0.94, CA: 1.48), IA predicted effects more accurately than CA. Because of its greater conservatism, CA can serve as a first-tier method in chemical risk assessment of metal-OMP mixtures. At a higher tier in chemical risk assessment, IA could be used because of its greater accuracy, provided that suitable data are available. Alternatively, the median MIF values could be used as correction factors to account for potential effect overestimations by CA and underestimations by IA.

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3.12.B.T-02 Beyond default Mixture Allocation Factor settings: increasing the applicability and scientific relevance of mixture risk assessments for metal(loid)s

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The impact of the combined exposure of chemical mixtures is receiving increased regulatory attention. For instance, within the Chemicals Strategy for Sustainability of the European Commission it is suggested that mixture effects will be covered by a default Mixture Allocation Factor (MAF), while also other environmental quality initiatives have considered additional safety margins. Analysis shows that the implementation of a default safety margins for the coverage of potential effects of combined exposure will have a large impact on the environmental risk assessment of inorganics (including metals and metalloids). Given that metal(loid)s are naturally occurring, the implementation of such safety margins may result in exceedances of safety limits even at natural background concentrations, suggesting the existence of combined risk. In addition, the theoretical assumptions underlying default mixture calculations and their associated conservatism are rarely evaluated. To anticipate the mixture toxicity correction in environmental regulations, a comprehensive study was set-up: the Metals Environmental Exposure Data (MEED) programme. This research programme focused on assessing existing knowledge on metal-mixture toxicity and addressing data gaps. The current study aims to summarize the learning lessons of the MEED-project in the context of risk assessment frameworks. This was done by quantifying the mixture risk associated with exposure to metal(loid)s in European freshwaters, based on monitoring data (default MAF). In addition, the conservatisms inherent to default mixture risk calculations were assessed. The conservatisms embedded in concentration addition (CA)-based risk calculations were quantified using the Margin of Safety (MoS) and the Mixture Interaction Factor (MIF). These quantify the deviation in mixture risk predictions of the assumptions I) that for all substances the same species drives the environmental threshold (MoS) and II) that all substances have the same mode-of-action (MIF). It was shown that default calculations overestimate mixture risks at the ecosystem-level on a median basis by 1.8-fold (MoS), while CA overestimates mixture toxicity of metals at the species-level by 1.3-fold (median MIF). A refined MAF should take into account the conservatisms quantified by the MoS and MIF. Supported by evidence on mixture toxicity, this refined MAF will increase the scientific relevance of mixture risk assessments for metal(loid)s.

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3.12.B.T-03 A Biological Line of Evidence to Validate the Zinc Environmental Quality Standard in Europe

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Under the Europe Water Framework Directive (2000/60/EC), Environmental Quality Standards (EQSs) are used to assess whether waters are meeting ‘good chemical and ecological status’. Technical Guidance is available to ensure the derivation of these EQS are evidence-driven and consistent. Biological field data can be used to validate newly derived or updated EQS. A new EQS value for Zn is proposed (11.2 µg/L), based on new bioavailability models and ecotoxicity data.

To validate its protectiveness, we analysed regulatory monitoring data (2012–2018) on freshwater macroinvertebrates in British waters using a “limiting function” approach. This method applies high-quantile regression to define the maximum achievable ecological quality at a given Zn exposure, accounting for multiple stressors. EC10 values were derived for the most sensitive taxa and compared to the proposed EQS using risk characterisation ratios (RCR).

Results show RCR values ?1 (1.03–1.26), indicating the revised EQS is protective of sensitive assemblages, though with a modest margin of safety. This field-based evidence provides a robust line of support for EQS derivation and aligns with EU Technical Guidance recommendations for uncertainty analysis. A new EQS value for Zn of 11.2 µg/L is concluded based on new bioavailability models and ecotoxicity data, which can be validated against field collected data on macroinvertebrate community responses.

3.12.B.T-04 Trophic Transfer of Lithium in Terrestrial Ecosystems

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Lithium (Li) is emerging as a potential environmental contaminant of concern due to its increasing global applications and production, yet it is unregulated and little is known about its bioaccumulation and trophic transfer potential in natural ecosystems. The only reported trophic transfer study of Li in complete field-based food webs was on marine ecosystems. In this work, we sampled soil, leaf litter, and ground-dwelling invertebrates from four temperate forests (the United States, US) and two subtropical forests (Hong Kong, HK), in which we analyzed their stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes to infer their energy sources and trophic positions, respectively. While we analyzed Li content for all samples. We found no significant differences in the Li levels of either soils or leaf litter between the US and HK forests ($p > 0.05$). Forest floor is clearly a net source of Li to the food webs, in which the decomposing leaf litter had Li concentrations 5- to 10-folds higher than fresh litter (Kruskal-Wallis; $p = 0.0012$). Our results demonstrated that Li bioaccumulation patterns in terrestrial ecosystems are primarily governed by the interplay between the diet and (specifically, leaf litter age), species traits (including growth stage and feeding habit), and to a lesser extent, the geographic location. For instance, by the tight coupling and consistent diminishing trend exhibited in both the $\delta^{13}\text{C}$ signatures and Li concentrations from fresh leaf litter to the strict herbivorous (i.e., Lepidoptera) order, evident in both the US and HK. This provided a clear soil-plant-primary consumer Li transfer pattern in terrestrial ecosystems for the first time. Also, by the order of median Li concentrations corresponding to leaf litter total Li contents’ differences (old > fresh leaf litter): detritivorous (e.g., earthworms; ~3,336.18 ng/g) > carnivorous, omnivorous > herbivorous > strict herbivorous (e.g., moths and butterflies; ~43.40 ng/g). While we observed trophic diminution in all forests, this pattern was only statistically significant ($p < 0.05$) in UMBS (US) and Tai Po Kau (HK) forests, and when all invertebrates (outliers included ~14 % of sample population) were considered. In summary, our study identifies the soil and decomposing litter as important sources of Li to the lower food webs through differential feeding habits. Li exhibited a weak potential in trophic transfer, which can mitigate its risk to top predators in the terrestrial biomes.

3.12.B.T-05 An approach to incorporate soil lead bioavailability in ecological risk assessment by including diet in a novel avian in vitro gastrointestinal assay

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A major challenge in ecological risk assessment is the lack of available avian toxicological data and the difficulty associated with obtaining it. Worst-case scenario estimates are often employed in situations where data is not available. When bioavailability data is not available for a chemical, it is often assumed to be 100% bioavailable, though many common contaminants of concern are in reality much less bioavailable. Overly conservative estimates of 100% bioavailability can thus result in overestimation of risk as well as unnecessarily cost- and time-intensive cleanups. Experimentally determining bioavailability directly is also unappealing as it necessitates in vivo studies which likewise raise cost and time concerns in addition to ethical ones. We report a novel in vitro method that can be used to accurately predict relative bioavailability of soil lead (Pb) to birds.

When diet is included in the novel in vitro method, in vivo-in vitro correlations were strong ($r^2 = 0.92$), showing potential for use in ecological risk assessment. Among six different avian diets, the in vitro bioaccessible Pb was strongly correlated with total phosphorus content ($r^2 = 0.78$). By including a representative diet sample in the in vitro assay, relative bioaccessible Pb can be accurately estimated while avoiding the need for animal experimentation or diet-specific adjustments for toxicity calculations. This relative bioaccessible Pb estimation can be used in ecological risk assessment to yield more true-to-life estimated exposure dose for comparison to avian toxicity reference values.

A bioavailability-based framework for avian ecological risk assessment will be presented.

3.12.P Fate and Toxicity of Metals: Recent Scientific Advancements and Their Application in Environmental Regulations

3.12.P-Mo166 Chronic Silver Toxicity to *Daphnia magna*: Development and Validation of a Bioavailability Model

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Widespread industrial applications of silver result in unavoidable releases to surface waters, where it exhibits toxicity to aquatic organisms even at sub- $\mu\text{g/L}$ concentrations. This raises regulatory concerns and underscores the need for scientifically derived environmental quality standards, which require an understanding of silver bioavailability across natural waters. Although an acute bioavailability model for silver exists, no chronic model is currently available, limiting our ability to assess long-term risks.

This study aimed to quantify the influence of key water chemistry variables on chronic silver toxicity to *Daphnia magna* and to develop a bioavailability model. Chronic 21-day reproduction tests were conducted in synthetic freshwaters while varying pH, calcium or magnesium concentrations across ranges representative of European surface waters. Ongoing tests will assess the influence of sodium and chloride, and tests in six natural freshwaters will further validate the model and provide insight into the influence of natural organic matter variability.

In synthetic freshwaters, calcium unexpectedly increased toxicity, reducing EC50s by a factor of approximately two (from 3.4 $\mu\text{g Ag/L}$ to 1.6 $\mu\text{g Ag/L}$) across the tested range (0.3 mM to 2.2 mM Ca), while magnesium showed no effect. Varying pH from 6.5 to 8.4 had no influence on chronic toxicity. Overall, the influence of water chemistry on chronic silver toxicity to *D. magna* was weaker than that commonly observed for other metals.

These results challenge classic assumptions in metal bioavailability modelling, particularly regarding cation competition at the biotic ligand. Ongoing validation in natural waters will determine model accuracy in natural water chemistries. The main relevance of this work lies in its applicability for regulatory purposes.

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3.12.P-Mo167 The Development and Regulatory Implementation of a Bioavailability Model for Uranium

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In parts of Europe, including Sweden, natural background concentrations of uranium can exceed the existing Environmental Quality Standard (EQS). It is widely recognised that many biological and chemical factors can influence the behaviour and ecotoxicity of metals, including uranium, in surface freshwaters. In line with bioavailability model developments in recent years for other metals (such as copper, nickel, zinc, cobalt) we are developing the first chronic bioavailability model for uranium in freshwaters. Here, we present our approach for

the development of this model, showing results from the initial testing, outline the likely way forward and discuss how the model will be used to fit with existing regulatory approaches to ensure improved evidence-driven risk assessment of uranium.

The model development is based on the results of long-term toxicity tests with algae, aquatic invertebrates and fish, which have investigated the influence of several toxicity modifying factors including pH, dissolved organic carbon (DOC) and hardness. The model will be validated with species not used in the development tests (but in the same respective taxa), in natural field-collected waters covering a wide range of water chemistry conditions. The development tests provide the model that then can be benchmarked against the validation tests.

The development testing for algae and Hydra have been completed, whilst the development testing for fish is still in progress, expected to be completed in early 2026. Once completed, the bioavailability model will be developed. In parallel, the validation testing for all three trophic levels is underway and due to be completed in 2026. Once the model is developed and validated, it will be finalised and then applied for normalisation of the species sensitivity distribution (SSD) for waters across Europe. From this, the 5th percentile hazardous concentration (HC5) will be identified for the most sensitive region (country) and from this the EQSbioavailable will be derived. A user-friendly tool for the model, based in Excel, will also be developed.

3.12.P-Mo168 Modelling the impacts of metals and acidity on benthic invertebrates in English and Welsh freshwaters

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Modelling the combined effects of metallic contaminants in the field represents a key challenge in metal ecotoxicology. We have previously developed the WHAM (Windermere Humic Aqueous Model)-FTOX β model quantifies combined impacts of cationic metals and acidity on biota and successfully applied it to time series recovery of lake zooplankton from past acidification and metal contamination. The purpose of this work is to apply the model to impacts in running freshwaters over a greater geographic range and to a broader expected set of significantly acting metals (Al, Mn, Ni, Cu, Zn, Cd and Pb). The model uses a dose term, FTOX, to quantify mixture effects. Metal-specific toxic potency coefficients (derived from laboratory toxicity testing) and taxon-specific sensitivity coefficients (derived from the laboratory or by optimization to field data) are combined with computed fractional occupancies of humic acid binding sites by protons and metals (computed using WHAM) to predict effects as an expected fraction of impacted taxa. We collected suitable water quality and benthic invertebrate taxon number data from 285 locations across England and Wales unimpacted by other chemical stressors and used the River Invertebrate Classification Tool (RICT) to predict the expected invertebrate taxon numbers at each location, allowing us to directly compare observed and modelled impacts. Application of a laboratory-derived distribution of organism sensitivity to metals predicted larger effects than were observed, at the most contaminated sites in the dataset. Optimization of the sensitivity distribution on the field data explained approximately half the observed variability in taxon numbers across the dataset. The optimized sensitivity distribution overlapped that derived from the laboratory but was notably broader. The model outputs allow assessment of the relative contributions of protons and each metal. Protons, Zn and Cu were the most frequent important contributors to impacts, while Cd was generally not important. There is considerable potential for further model application, for example to identify taxonomic sensitivities directly from field data.

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3.12.P-Mo169 Effects of Water Chemistry on Cobalt, Copper, and Zinc Toxicity in Arctic-acclimated *Daphnia pulex*

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In an effort to reduce climate change-associated impacts, the extraction of critical minerals used in green technologies is increasing globally. Sensitive environments, such as the Arctic, which contain large deposits of minerals, have an increased risk due to the region's unique physicochemical properties. Low temperatures and large variability in water hardness and pH can dictate the mobilization and speciation of metals within the environment. Furthermore, Arctic organisms possess a slower metabolic rate, extended lifespans, and limited

biological activity compared to their temperate counterparts, altering their susceptibility to metal contaminants. Current government-defined water quality guidelines are based on lower latitude organisms which may not be representative of Arctic systems. This study uses cold-adapted *Daphnia pulex*, a crustacean inhabiting both Arctic and temperate freshwater bodies, in Arctic-relevant water to determine the effects of copper (Cu), cobalt (Co), and zinc (Zn). The lethal concentration 50 (LC50) was assessed at two temperatures (8°C and 20°C) and three pHs (7.0, 7.6, and 8.2) under site-specific water hardness (10 mg/L calcium and 5 mg/L magnesium). The highest Cu toxicity was observed at pH 7.0 with an LC50 value of 10.4 µg/L (95% CI 8.59, 13.3) at 20°C and 9.86 µg/L (95% CI 9.25, 10.7) at 8°C. Arctic-acclimated *D. pulex* were also significantly more sensitive to Cu in the 8°C pH 8.2 group, with an LC50 value of 16.8 µg/L (95% C.I. 20.3, 29.5) compared to 24.2 µg/L (95% C.I. 20.3, 29.5) at 20°C. For Co, a decrease in pH unexpectedly decreased toxicity indicating the metal may have formed a complex bioavailable to the organism. Values ranged from 2.05 mg/L (95% C.I. 1.79, 2.38) for pH 7.0, and 1.11 mg/L (95% C.I. 0.97, 1.26) for pH 8.2, both at 20°C. At 8°C, using the same Co range, no LC50 values were able to be determined indicating the 48-hour test duration may be insufficient for Arctic species. Metal bioaccumulation of *Daphnia pulex* will also be measured at the two temperatures and three pHs across various timepoints (48, 96, 120, and 144 hours for 8°C and 48 hours for 20°C) to investigate potential differences in uptake rate and to determine an equivalent timepoint at 8°C. Understanding the toxicity of critical minerals using cold-adapted organisms within their relevant water chemistry will help develop Arctic-specific risk assessment models.

3.12.P-Mo170 Environmental Hazard Assessment of Cadmium in Soil. A Comparison between Toxicity to Soil Organisms and Secondary Poisoning to Wildlife.

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A comprehensive update of the hazard assessment of cadmium (Cd) contamination in soils was conducted, including both ecotoxicological effects on soil-dwelling organisms and secondary poisoning risks to wildlife from soil. Reliable chronic toxicity data were identified for 42 terrestrial plant species, 15 invertebrate species and 11 microbial endpoints. Chronic EC10 and NOEC values vary between 0.5 and 4596.9 mg Cd/kg dry weight (dw). Although soil properties such as pH and organic carbon influence Cd bioavailability, species-specific sensitivity was the dominant source of variation. The data were evaluated using a species sensitivity distribution (SSD), which gave a 5th percentile hazardous concentration (HC5) of 2.18 mg Cd/kg soil for direct effects. Available field data on plant growth in Cd contaminated soils confirm that this laboratory-derived threshold is protective under realistic conditions. The assessment of secondary poisoning of Cd to wildlife via predicted exposure in food is complex and the standard assumptions on soil-earthworm-predator pathway and Cd availability in diet can result in unrealistic results. Since the kidney is the critical organ for Cd toxicity in the field, an alternative approach based on critical Cd concentrations in kidneys of mammals and birds in relation to soil Cd concentrations of their foraging area overcomes these assumptions. The critical soil Cd concentration is defined as that concentration at which a critical kidney Cd concentration of 400 mg/kg dw (whole kidney) is predicted using a proportional extrapolation. Paired sets of soil Cd and kidney Cd were found for 23 mammal species and 3 bird species. Moles have the lowest predicted critical Cd concentration in soil (0.6 mg/kg dw), while deer mice have the highest predicted critical Cd concentration in soil (1813 mg/kg dw). The 5th percentile of predicted critical soil concentrations was 2.05 mg Cd/kg soil, i.e., slightly below the threshold for direct toxicity of soil Cd to soil dwelling organisms showing that secondary poisoning is indeed a critical pathway to consider for addressing the risks of cadmium contamination in soil. Overall, these thresholds exceed typical ambient background concentrations, with only ~1% of European and North American soils surpassing 2 mg Cd/kg, suggesting limited ecological risk under current ambient soil Cd levels.

3.12.P-Mo172 Different Coat, Different Interaction? Effects of Differently Coated Silver Nanoparticles on Freshwater Cyanobacterium *Synechocystis* sp. PCC 6803

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Due to their antibacterial properties, silver nanoparticles (AgNPs) represent the fastest growing class of engineered nanomaterials. The increased production and widespread use of AgNPs have raised the likelihood of these particles entering the aquatic ecosystem through accidental release or leaching. Even though the effects of engineered nanomaterials within the aquatic environment have become a subject of concern, there remains a lack of research on the impact of nanoparticle surface coatings on cyanobacteria. To address this gap, two different initial cell concentrations of *Synechocystis* sp. PCC 6803 were exposed to increasing concentrations (0–100 µg/L) of (1) 50 nm citrate-coated AgNPs and (2) 50 nm PVP-coated AgNPs. Growth inhibition was

monitored every 24 h up to 72 h via flow cytometry, while photosynthetic efficiency and photoprotection were assessed through pulse-amplitude modulated fluorometry at the 72 h. Growth inhibition was more pronounced in cultures exposed to citrate-coated AgNPs compared to equivalent concentrations of PVP-coated AgNPs, while relatively higher sensitivity was found in cultures with lower initial cell densities. Photosynthetic efficiency declined with increasing AgNP concentrations, whereas photoprotective activity rose. By examining how nanoparticle coating modulates toxicity towards primary producers in freshwater systems, the study enhances the understanding of environmentally relevant modes of action and supports more accurate ecological risk assessments of AgNPs.

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3.12.P-Mo173 Exploring Dose-Dependent Effects of Copper Oxide Nanoparticles and Dissolved Copper on Photosynthesis and Growth in Freshwater Microalgae

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Copper oxide nanoparticles (CuONPs) are increasingly released into aquatic systems, yet their dose-dependent and sublethal effects on algal photosynthesis remain insufficiently characterised. In particular, low-dose exposures are rarely examined, which may obscure potential stimulatory or hormetic responses. This study aims to investigate the effects of a broad concentration range (0.001-100 mg L⁻¹) of 40 nm CuONPs and dissolved copper on freshwater microalgae *Raphidocelis subcapitata* and define if and under what conditions a biphasic stimulatory/inhibitory dose-response can be observed. Cultures were exposed to a wide range of copper concentrations, from low to high doses. The effects of CuONPs and ionic copper were then compared to determine whether they elicited similar or distinct impacts on photosynthesis and growth. Photosynthetic responses were assessed using chlorophyll fluorescence measurements obtained with a Pulse Amplitude Modulation (PAM) technique. Key parameters, including maximum quantum yield, photochemical and non-photochemical quenching, were evaluated to capture changes in both efficiency and protective regulation of photosystem II. Growth and pigment autofluorescence were monitored to complement fluorescence-based endpoints and provide integrative information on physiological state at the single-cell level. Nanoparticle behaviour in the exposure medium, including aggregation and dissolution, was quantified to link physicochemical properties to biological responses. The results revealed significant growth inhibition and effects on the photosynthetic apparatus following exposure to CuONPs and Cu²⁺ at high concentrations. By examining nanoparticulate and dissolved copper across a broad range of exposure, this study provides mechanistic insight into their effects on algal photosynthesis and growth. These findings will enhance our understanding of subtle, sublethal responses relevant to the ecological risk assessment of metal oxide nanoparticles.

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3.12.P-Mo174 Uranium toxicity to algae and water flea in natural freshwaters. Validating Biotic Ligand Model for uranium.

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Water chemistry significantly influences the bioavailability of metals in freshwaters. Bioavailability models have been developed for several metals, such as Cu, Ni, Zn, and Co, and are used to derive safe surface water

concentrations. All models must be validated through toxicity tests conducted under various natural water chemistry conditions. Typically, three trophic levels (algae, invertebrates, fish) are selected for these tests. The test-based effect concentrations are then compared to the model-based concentrations during the validation process.

Our objective is to generate toxicity data that will be used to validate a chronic bioavailability model for uranium in freshwaters. We collected five natural water samples from Finland and Sweden, representing the water chemistry conditions under which the model was developed. The standard test species chosen include green algae (*Raphidocelis subcapitata* SAG 61.81 strain originally from Norway) and water flea (*Daphnia longispina*, sourced from a nature conservation area in Finland). These organisms will be exposed to uranyl nitrate hexahydrate ($\text{UO}_2(\text{NO}_3)_2$) in chronic tests. Non-linear regression models will be employed to estimate the effective concentrations (EC10) for algae growth rates and water flea reproduction. The results are expected to be available early 2026.

3.12.P-Mo175 Metal accumulation and Metallothionein responses in the Mediterranean invasive blue crab (*Callinectes sapidus*) across salinity regimes

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The blue crab (*Callinectes sapidus*), an invasive species expanding across Mediterranean coastal lagoons, tolerates salinities from near-freshwater to hypersaline. In invaded ecosystems, it alters food webs as an omnivorous top mid-predator, scavenger, and prey, including as a human seafood resource. Salinity strongly influences metal bioaccumulation and detoxification in aquatic organisms, yet the physiological mechanisms regulating ion transport, gill permeability, and metabolism in highly euryhaline crustaceans remain poorly understood. Salinity also shapes prey communities, modifying trophic exposure, so metal bioaccumulation reflects both environmental chemistry and food web shifts. Essential metals are physiologically regulated, whereas non-essential metals accumulate with exposure. Metallothioneins (MTs), cysteine-rich proteins key to metal detoxification and sequestration, mediate metal homeostasis. In *C. sapidus*, multiple MT isoforms bind Cu, Cd, Hg, and Zn. Establishing baseline MT levels across salinity regimes is essential to accurately interpret metal exposure and detoxification in this euryhaline species.

We measured metals (Cd, Cu, Pb, Zn, As, Ni, Cr, Co, Mn, Tl) and MTs in *C. sapidus* from two contrasting Mediterranean ecosystems: the hypersaline Mar Menor lagoon and low-salinity El Hondo wetland. Metals were quantified in gills, hepatopancreas, haemolymph, and muscle; MTs were measured in gills and hepatopancreas. We analyzed spatial, tissue-specific, and sex-related patterns, and correlated metal and MT levels with physicochemical and biological variables.

This study investigates the role of blue crabs as vectors of metal contaminants across coastal ecosystems and assesses ecological risks and human exposure through consumption. The use of MTs as biomarkers of metal exposure is evaluated, highlighting their potential in integrated environmental monitoring programs in Mediterranean ecosystems affected by invasive species.

Disclaimer/Disclosure:

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3.12.P-Mo176 One lake, two worlds? – Mercury dynamics in food webs from two basins in human-impacted Lake Vesijärvi, Finland

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Clean lakes and resilient food webs are vital to maintaining biodiversity and ecological integrity, however, pressures on these important ecosystems are numerous. The VesiMon project aims to develop and test methods to holistically understand aquatic ecosystems, identify pressures on them, and make the monitoring of water bodies more accurate and cost-effective. In this study, primary producers, pelagic and benthic invertebrates, and the fish community were sampled in the Enonselkä and Kajaanselkä basins of Lake Vesijärvi in southern Finland. These basins represent different levels of productivity and human-impact, with the Enonselkä basin

being more eutrophic due to a history of municipal sewage inputs. The aquatic community of these basins was assessed regarding stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$), total mercury content [THg] (mg/kg) and bioaccumulation of THg in different fish species, and biomagnification of THg through the entire food web. The [THg] was highest in piscivores and generalists, and lowest in benthivores and planktivores, without consistent significant differences detected between the basins. Bioaccumulation regression slopes between [THg] and fish length were most elevated in planktivorous fish in both basins, and species-specific differences between basins were apparent. Biomagnification of THg, defined as the regression slope between [THg] and $\delta^{15}\text{N}$, was significant in both basins but did not differ significantly between them. Stable isotope analyses indicated higher $\delta^{13}\text{C}$ in the Kajaanselkä basin and higher $\delta^{15}\text{N}$ in the Enonselkä basin, thus indicating the higher nutrient status of the latter basin. Overall, THg analyses indicated that although variations in [THg] and bioaccumulation were apparent in fish, these differences were more closely related to species characteristics than to any systemic differences between the basins. While these methods provided a wealth of information on the food webs of these basins, the basins are notably similar in mercury metrics, while differences in stable isotope ratios represent the shifting energy sources of the Enonselkä basin.

Disclaimer/Disclosure:

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3.12.P-Mo177 Challenges in Applying Metal Environmental Quality Standards in Freshwater: Estimation of Natural Background Concentrations in Flanders.

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In Flanders, the Flemish Environmental Agency has developed environmental quality standards (EQS) for various metals in freshwater systems. The derivation follows the methodology outlined in the EU Technical Guidance Document No. 27 (2018). Metals represent a particular challenge in the derivation and application of EQS due to their natural occurrence in the environment, their essentiality for certain species, and their complex behavior as function of physico-chemical properties in aquatic systems. As a result, metal-specific EQS values are often associated with considerable uncertainty.

To address these issues, EU Guidance Document No. 38 (2019) provides additional recommendations for deriving metal EQS, including the consideration of natural background concentrations (NBCs).

Based on this guidance document, we estimated regional NBCs for several metals (arsenic, barium, cobalt, iron and vanadium) in Flemish freshwater ecosystems. The application of these NBCs and their implications within the EU Water Framework Directive are discussed.

3.12.P-Mo178 Zinc in German Freshwaters: Searching for Ecological Signals in Real-World Monitoring Data and Evaluation of a Bioavailability-Based Environmental Quality Standard (EQS)

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Zinc occurs naturally in freshwater systems and is essential for many biological processes, but higher concentrations can lead to ecotoxicological effects. Zinc has numerous applications across many industries and, as such, it is critical to understand the potential risk of increased concentrations of zinc to environmental health. One key regulatory tool to support safe use of substances such as zinc is an environmental quality standard (EQS) compliance assessment. A bioavailability-based EQS for zinc has been derived for regulatory use in Europe, yet there is still relatively little information on how well real-world monitoring data reflect possible ecological responses to zinc. In this project, we examine whether existing German monitoring datasets offer any evidence of zinc influencing biological communities, particularly at or around the bioavailable-EQS.

To do this, we brought together bioavailable zinc measurements from several German Länder and combined them, where possible, with corresponding biomonitoring data for fish, macrozoobenthos, phytoplankton and macrophytes/phytobenthos. The focus of the analysis is on exploring patterns in community metrics in relation to zinc concentrations, recognising the challenges posed by combining data from different monitoring programmes. This required harmonising disparate datasets, aligning sampling locations, and applying spatial and temporal matching rules to ensure that chemistry and biology data were meaningfully comparable. Where the data allows, community metrics such as richness, abundance, and other established assessment indices are examined in relation to bioavailable zinc concentrations and acknowledging (or where possible, accounting for)

the presence of multiple environmental pressures. The study offers a weight-of-evidence perspective that may inform on the protectiveness of the bioavailable Zn EQS to real world communities.

3.12.P-Mo179 Metalloids Monitoring in Offshore Produced Water: Analytical Insights and Environmental Implications

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Produced water (PW), the largest waste stream from offshore oil and gas production, is almost entirely discharged to the sea. It contains complex organic and inorganic pollutants, including toxic metalloids such as mercury (Hg), arsenic (As), antimony (Sb), and selenium (Se). These elements pose ecological and human health risks, particularly in fish-rich marine environments like the North Atlantic. Despite their relevance, comprehensive temporal data on metalloid concentrations in North Sea PW are lacking. Analytical quantification is challenging due to PW's high salinity (~70‰), hydrocarbons, and production additives, which can interfere with conventional measurement techniques. For this reason, the presented study evaluates cold vapor atomic fluorescence spectrometry (CV-AFS) and inductively coupled plasma mass spectrometry (ICP-MS) for ultra-trace metalloid detection in PW and presents a multi-year dataset (2022–2024) from four mature North Sea chalk oilfields.

Field-average Hg measured in PW by CV-AFS exceeded both North Atlantic background levels and predicted no-effect concentrations (PNECs). This was not in agreement with commercially reported values (often obtained with standard ICP methods). Concentrations indeed varied across fields and throughout time, influenced by production practices, sampling approaches, and matrix complexity. However, CV-AFS demonstrated superior sensitivity for hydride-forming elements, minimizing over- or underestimation caused by ICP-MS matrix interferences. Risk assessment revealed that instantaneous PEC/PNEC ratios for Hg and Se exceeded 1 in multiple fields, indicating potential localized ecological impacts even at moderate discharge volumes. Correlations with outlet temperature and oil-in-water content suggest that current discharge controls do not sufficiently mitigate metalloid risk.

The results emphasize the critical need for optimized sampling protocols, acid-washed glassware, airtight seals, and rapid analysis to reduce variability and improve analytical accuracy. Integration of metalloid monitoring with existing oil-in-water discharge regulations is recommended to strengthen environmental risk management of offshore PW. By providing validated analytical methods and evidence-based risk insights, this study supports regulatory decision-making and operational practices to mitigate metalloid impacts in marine environments.

3.12.P-Mo180 Photochemical, Abiotic, and Microbial Pathways of Mercury Reduction in Aquatic Environments

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Mercury (Hg) is a global health concern due to the long-range intercontinental transport of elemental Hg (Hg(0)) in the atmosphere and microbial methylation of inorganic Hg to produce highly toxic and bioaccumulative methylmercury in aquatic environments. Biogeochemical cycling of Hg in the environmental media (air, soil, water, and sediment) involves various transport and transformation processes, among which reduction of divalent Hg (Hg(II)) to Hg(0) in aquatic environments is a critical step as it controls the air-water Hg exchange and the pool of inorganic Hg available for methylmercury production. Photochemical reaction has been extensively studied as a dominant pathway for the reduction of Hg(II) to Hg(0) in sunlit environments such as surface water. The reduction of Hg(II) in the environment with limited light penetration (e.g., aphotic water layer and sediment) can be attributed to microbial and dark chemical processes. It is well known that Hg-resistant bacteria can use the MerA pathway to reduce Hg(II), which is usually mediated by aerobic heterotrophic microbes in Hg-contaminated areas. In anaerobic zones, Hg(II) reduction can occur through the activities of some heterotrophic and chemotrophic microorganisms, e.g., the dissimilatory iron (Fe) reduction bacteria. The widely present ferrous Fe (Fe(II)) species, including aqueous Fe(II) and especially solid phase Fe(II) such as magnetite, green rusts, and mackinawite, may be a critical driving force for dark chemical reduction of Hg(II), although their exact roles in Hg redox transformation in aquatic environments warrant further investigation. We present here a comprehensive review of aquatic Hg(II) reduction by synthesizing previous information and highlighting future research needs.

3.12.P-Mo181 Organometallics in Commerce: A Scoping Review of Environmental Fate and Behavior

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In recent years, the synthesis and assessment of chemical inventory data have greatly advanced our understanding of the composition of the global chemical market. A landmark contribution to this effort was made by Wang et al. (2020), who compiled a unified global chemical inventory encompassing over 350,000 chemicals and mixtures. Within this landscape, organometallic compounds emerge as a particularly significant class of chemicals, with over 10,000 identified.

Evidence from well-studied examples, like those containing lead, mercury, and tin, demonstrates that many of these substances can be persistent, bioaccumulative, and toxic. Yet only a limited number of organometallics appear to have been thoroughly studied or publicly assessed with respect to their environmental fate and behavior, with existing information often highly fragmented. In response to this situation, this poster presents the first structured overview of data relevant for the environmental assessment of organometallics in commerce.

Using structural filters, we extracted 10,613 organometallic substances from the latest version of the global chemical inventory and assembled a PostgreSQL database integrating data from 25 regulatory, scientific, and industrial sources. CAS Registry Numbers were used as the common identifier to link inventory chemicals to the respective sources. The final database includes harmonized structural identifiers, production and use information, hazard classifications, and available physicochemical properties. Using these data, we further characterized and mapped the chemical space of organometallics through cheminformatic analyses and categorized substances based on use patterns and hazard classifications wherever possible.

Our findings point to substantial data gaps across all dimensions investigated, with information heavily skewed toward main-group organometallics. Even so, we identified approximately 600 substances of potential concern, including several high-production-volume chemicals used in applications with high exposure potential. In contrast, only a small fraction of organometallics could be sufficiently characterized in terms of their properties, hindering environmental assessment for most substances. Overall, our work highlights organometallics in commerce as an overlooked systemic issue and identifies strategic needs that require attention from both researchers and policymakers to advance future evaluation and regulatory decision-making.

3.12.P-Mo182 Isotope-Tracing of Tin Methylation and Degradation in Marine Sediments

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The methylation of tin (Sn) is environmentally significant because it converts inorganic, non-toxic and low-mobility Sn species into soluble, mobile and harmful methyltin compounds (MeTs). Trimethyltin (TMT) is a particularly potent neurotoxin widely used as a model compound in neurotoxicity studies. Despite their relevance, the formation pathways and environmental behavior of MeTs remain poorly understood, and their concentrations in marine environments are rarely reported. Although MeTs are known to occur in seawater and sediments, in situ evidence of Sn methylation is scarce, making it unclear whether detected MeTs originate from natural processes or anthropogenic inputs.

This study investigated the formation and degradation of MeTs in 16 marine sediments under oxic and anoxic conditions. Sediments were spiked with isotopically enriched inorganic Sn (Sn119) and enriched mono- and dimethyltin (MMT116, DMT116). Transformations were monitored over 180 days. Comprehensive sediment characterization (grain size, mineralogy, elemental composition, TOC, carbonate content) and amplicon-based microbial community profiling were performed to identify factors controlling MeTs dynamics. Transformation rate constants were calculated from concentration changes over time.

The results showed that MMT116 and DMT116 degraded efficiently in all sediments, decreasing by 20–40% over 180 days, with faster degradation under oxic conditions. Formation of TMT116 through methylation of MMT116 occurred only in one anoxic sediment (K-A), reaching 11 ng/g after 180 days. Although the first step of inorganic Sn methylation to MMT was not observed, further methylation to DMT was detected exclusively in sediment MAN-O, where DMT119 formed under oxic conditions after 180 days. MAN-O is a partially oxygenated clayey silt with moderate organic matter and microbial communities including early-stage sulfate-reducing bacteria, enabling a short “hot moment” of methylation. In contrast, K-A is a sandy silt that developed strongly reducing conditions and a consortium dominated by metal- and sulfate-reducers, fermenters and methanogens, enabling efficient methylation.

Overall, the results demonstrate that Sn methylation can occur in marine sediments, although degradation

generally prevails. Nonetheless, under specific sedimentological and microbial conditions, toxic MeTs, including TMT, may form.

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3.12.P-Mo183 Stress Responses and Toxicity Associated with Exposure of *Acanthamoeba castellanii* (Amoebozoa; Acanthamoebidae) to Trace Metals

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Despite their natural occurrence in the environment, many metals are known to become toxic to organisms at elevated concentrations. With changes in industrial processes and technological developments, novel elements are now being released into aquatic ecosystems. Free-living protists, such as amoebae, perform critical roles in aquatic environments as both bacterial grazers and microbial reservoirs, including potential pathogens. Few studies, however, have compared the toxicity and responses of protists to metals routinely released into natural environments through anthropogenic activity. This is despite some metals such as silver being well-known for having antimicrobial properties. The present study aimed to compare the toxicity and morphological changes in the free-living protist and opportunistic pathogen, *Acanthamoeba castellanii* when exposed to aluminium, silver, and vanadium metals. Exposures were carried out under controlled conditions for two replications. *Acanthamoeba castellanii* (ATCC 50370) were cultured in peptone glucose medium and exposed to varying trace metal concentrations. These metals were chosen given their use in new and developing technologies such as renewable energy storage. All metals induced a dose-dependent response in *A. castellanii*, characterised by increased mortality and enhanced cyst formation. Mortality was quantified using Alamar Blue assays and verified through morphological assessment, while mature cysts were stained using Calcofluor White. These results indicate that *A. castellanii* responds to metal stress through encystation, enabling survival under otherwise toxic conditions. Such shifts towards cyst dominance could disrupt microbial interactions, alter grazing dynamics, and ultimately influence wider ecosystem functioning in metal-impacted environments.

Disclaimer/Disclosure:

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3.12.P-Mo184 Uranium Toxicity to Zebrafish in Reconstituted Freshwater at Different pH and Ionic Composition

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Water quality characteristics influence the aqueous bioavailability and hence toxicity of many metals and radionuclides, including uranium. Past research has shown that pH is among the most relevant variables in those relationships. Additional variables such as water hardness, alkalinity and dissolved organic carbon can also play important roles. However, surprisingly little is known about the toxicodynamic relationships between these water quality parameters and uranium in key aquatic receptors, hampering environmental risk assessment efforts and guidelines development. This research project is part of a larger effort to generate the data needed to develop a generalized bioavailability model for uranium toxicity to aquatic organisms. The research presented here will focus on the chronic toxicity of uranium to zebrafish (*Danio rerio*), a model test species, at different aqueous pH levels. Specifically, zebrafish are sub-chronically exposed to incremental concentrations of uranium for 28 d starting with 724 hr old embryos. Primary biological endpoints include hatching success, survival at swim-up (approx. day 6), and survival, growth and teratogenicity at 28 d. Tests are conducted at six pH levels

(6.0, 6.5, 7.0, 7.5, 8.0, 8.5) covering a range of values representative of natural waterbodies. All tests are conducted in reconstituted freshwater with average hardness and alkalinity of approx. 50 mg/L as CaCO₃ each and no DOC. Four additional tests will be conducted with different concentrations of Ca, Mg and DOC. Tests conducted at very low alkalinity proved unsuccessful due to a reduced ability at maintaining dissolved uranium (added as uranyl nitrate) at nominal levels. Tests conducted to date show that uranium toxicity to early life stage zebrafish falls in the 0.2 to 2.0 mg U/L range (hatching success and lethality). Preliminary data also suggests that there is an influence of pH on U toxicity. Additional results on survival, growth and developmental deformities across the full suite of water quality variables investigated will be presented (experimentation still in progress).

Disclaimer/Disclosure:

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3.12.P-Mo185 Ecotoxicity of Environmentally Realistic Metal-Organic Mixtures to the Freshwater Algae *Raphidocelis subcapitata*

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Environmental risk assessment frameworks still predominantly follow a substance-by-substance approach, even though chemical contamination in the environment typically occurs as complex and often unknown mixtures. Regulatory agencies are increasingly recognising the need to assess the risks associated with chemical mixtures and to systematically integrate combined exposure and effects into chemical risk assessments. In this study, we investigate the aquatic toxicity of environmentally realistic mixtures containing both metal and organic contaminants.

We selected four representative real-world mixtures from the European freshwater monitoring database, focusing on combinations of five metals and four organic compounds. Monitoring database (EU Waterbase) analysis showed that five metals and five organics were sufficient to explain 90% of the overall mixture risk in 90% of samples. For each of the four metal-organic mixtures, we conducted 72-hour growth inhibition tests with the freshwater algae *Raphidocelis subcapitata*, following the OECD 201 guideline. We first performed range-finding tests to estimate the toxicity of the reconstituted metal mixtures and organic mixtures by testing them at different relative enrichment factors (REF). The REF is the metric used to express the concentration or dilution of a mixture of contaminants relative to their environmental levels, while maintaining their relative proportions constant. We then conducted binary mixture toxicity tests to assess any interactions between the metal and organic mixture groups.

Our specific experimental design aimed to address 2 main research questions: i) which mixture model, independent action or concentration addition, predicts more accurately the toxicity of environmentally realistic metal-organics mixtures, and ii) whether complex organic mixtures present below no-effect concentrations influence metal toxicity, and vice versa. If no influence is observed, it would suggest that metals and organic chemicals could be assessed independently in regulatory frameworks; vice versa, if an influence is observed, it would suggest that they should be considered jointly.

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 in aquatic environments.

3.12.P-Mo186 Influence of Environmentally Realistic Organic Micropollutant Mixtures on Metal Mixture Toxicity: Toward More Ecologically Relevant Risk Assessment

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Substance-by-substance risk assessments may underestimate mixture or combined exposure effects. Hence, a mixture assessment factor (MAF) is proposed as a default approach to address potential mixture effects, particularly given the limited understanding of how environmentally realistic organic micropollutant (OMP) mixtures may influence metal toxicity.

This study investigates whether an organic-metal MAF is needed and seeks to quantify its value by conducting 21-day reproduction tests with *Daphnia magna* (OECD 211). If realistic organic mixtures do not influence the toxicity of realistic metal mixtures, this could indicate that organics and metals can be regulated independently

within environmental risk assessment frameworks in respect to mixture toxicity.

To construct realistic exposure scenarios, metals in river samples were prioritized to reconstitute a quinary metal mixture representing environmentally relevant conditions. Organic compounds were extracted from wastewater treatment plant (WWTP) effluents using large-volume solid-phase extraction (LVSPE) to obtain a representative organic mixture. The metal mixture concentration series were then combined with varying amounts of this organic extract to quantify potential interaction effects. The experimental setup was replicated for 3 different river systems in Flanders: Dommel, Grote Struisbeek and Grote Nete.

Preliminary comparisons of metal mixture dose–response curves with various levels of added OMP indicate that organic mixtures exerted slight antagonistic to negligible effects on metal mixture toxicity. This interaction was both location - and concentration-specific. In most treatments, no significant influence of the OMP mixture was observed; only at certain organic–metal concentration combinations did a modest antagonistic effect occur. A possible explanation is that organic compounds in general may form complexes with metals, thereby reducing their bioavailability and mitigating toxicity.

Based on these findings, a specific organic–metal mixture assessment factor (MAF) does not appear necessary. Thus, LVSPE-extracted OMP influences on metal mixture toxicity may be considered negligible within the current environmental risk assessment framework for metals. Further replication of this experiment with additional test species and targeting other organics is required to validate whether the observed mechanism applies across multiple trophic levels and a broader set of organic-mixtures.

Disclaimer/Disclosure:

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3.12.P-Mo187 Street-Level Contamination: Heavy Metal Pollution and Human Health Implications in South African Urban Soils and Sediments

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Highways are major sources of inorganic contaminants in urban environments, particularly in South Africa, where poor road conditions, heavy traffic, engine emissions, overloaded vehicles, industrial activities, and inadequate waste management contribute to elevated heavy metal pollution. These pollutants accumulate in street-level soils, creating exposure pathways like dermal contact, inhalation, and ingestion that pose significant health risks to pedestrians and street vendors. This study investigated the concentrations of priority heavy metals (As, Cd, Cr, Cu, Pb, Hg, Zn, Mn, Co, and Ni) in urban roadside soils from Johannesburg, Randburg, and Thohoyandou, selected to represent contrasting levels of traffic density, industrialization, and urban development. Results revealed unexpectedly high contamination levels in shaded soils of Thohoyandou, which, despite being a moderately trafficked and less industrialized town, recorded the highest concentrations of Pb (2261.98 mg/kg), Cr (179.23 mg/kg), Cu (260 mg/kg), Mn (899.81 mg/kg), and Co (44.35 mg/kg). Conversely, unshaded soils of Johannesburg displayed the highest Ni (330.05 mg/kg) and Hg (1762.41 mg/kg) levels, consistent with its intense urban and industrial activity. These findings indicate that heavy metal pollution is not solely driven by large-scale urbanization but can also be exacerbated by rapid town development, unregulated vehicle conditions, and inadequate environmental management practices. The elevated metal concentrations observed suggest potential ecological and human-health risks that warrant urgent attention. We recommend periodic monitoring of roadside soils, enforcement of vehicular emission standards, improved waste-management strategies, and targeted risk assessments for vulnerable populations living or working near busy roadways.

3.12.P-Mo188 Research on Source Analysis and Background Value Determination of Heavy Metals in Soil of Lead Zinc Mining Area in Southern Gansu Province

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In order to scientifically determine the background values of heavy metals in the soil of the Chengxi lead-zinc ore belt in southern Gansu, this study systematically collected multiple representative soil samples from the region and measured the contents of seven heavy metal elements, including As, Cd, Cu, Hg, Ni, Pb, and Zn. We adopted various internationally recognized methods such as traditional mathematical statistics, median $\pm$ 2MAD method, normalization processing method, and cumulative frequency curve method, and based on this, we developed a "robust iteration geochemical method" to comprehensively determine the soil background value range of each element. The sources and spatial differentiation characteristics of heavy metals were explored through correlation analysis and principal component analysis (PCA). The results indicate that the content of

Cd, Pb, and Zn in the soil of the study area is significantly higher than the national soil background values, showing obvious geochemical characteristics of mineralization. Correlation analysis and principal component analysis revealed three main sources of heavy metals: associated elements of lead-zinc mineralization (Cd, Pb, Zn), parent material sources of soil formation (Ni, Cu, As), and independent high background sources of mercury. The consistency of the background value range determined by multiple methods is strong, among which the new method is recommended as the best representation of the background value in the region due to its robustness. This study provides important benchmark data and scientific methods for accurate evaluation of environmental quality, pollution risk assessment, and ecological restoration in high background value areas.

3.12.P-Mo189 Copper Pollution in the Arctic - An Aggregate Exposure Pathway for Understanding Sources, Sinks, and Bioavailability

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Copper is both a vital nutrient and material in biota and industry, and a classical stressor for wildlife and the natural environment. Growing demand for copper and increasing Arctic industry will likely increase both copper emissions and concurrent stressors. A more comprehensive understanding of pollutant dynamics will aid academics and regulators in understanding pressures on this sensitive and rapidly changing region.

Aggregate Exposure Pathways (AEPs) provide a comprehensive, weight-of-evidence based framework for characterising chemical exposure to wildlife of interest. Although copper is among ecotoxicology's most studied pollutants, an overall assessment of copper pollution in the Arctic has not been conducted. AEPs are well suited to mapping the profile of Arctic copper pollution thanks to their support for synthesis of available literature and regulatory data with weight-of-evidence assessment. Thus, information on occurrence and dynamics can be integrated into a source to dose pathway description that identifies current knowledge and data gaps.

We present our work on developing an AEP for copper pollution and exposure to wildlife in and around the Arctic circle, based on a systematic review of scientific literature and an assessment of Norwegian environmental monitoring data. We assess sources, receiving compartments, flows, and biota exposure to copper in our qualitative based mapping. By comparing measured concentrations to background levels, we establish a threshold above which chemical analysis data can be considered evidence of contamination. By applying the Criteria for Reporting and Evaluating Exposure Datasets (CREED) to evidence lines we quantify the confidence in each line of evidence.

Our work advances the understanding of copper pollution in Scandinavia and the AEP concept, integrating best practice in exposure assessment, systematic literature, and weight-of-evidence assessment to take the paradigm to its next logical step. Additionally, it will serve as the basis for quantification and modelling of copper fate that can be used for environmental risk assessment as part of the Source to Outcome Pathway (STOP) concept.

Disclaimer/Disclosure:

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3.13.A Harnessing Nature: Innovative Bioremediation and Phytotechnologies for Contaminant Removal and Ecosystem Recovery

3.13.A.T-01 Nature's Clean Up Crew: A System's Approach to Halogenated Contaminant Biotransformation

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Emerging contaminants, including per- and polyfluoroalkyl substances, pesticides, and legacy chlorinated solvents continue to persist and accumulate across ecosystems. Existing abiotic treatment technologies are costly, energy-intensive, require toxic inputs, produce secondary waste, and often incomplete in mixed-contaminant settings. Bioremediation research has historically focused on bacteria, leaving fungal pathways under explored despite their known oxidative and reductive enzymatic capabilities. This study addresses this gap by evaluating filamentous fungi as nature-based biocatalysts for halogenated contaminant transformation and establishing an integrated experimental-mechanistic-genomic workflow to characterise fungal dehalogenation capacity.

Bench-scale microcosms were constructed using white-rot and dematiaceous fungi exposed to halogenated nitrophenols as model substrates. Transformation products were monitored using GC–MS for volatile metabolites and ion chromatography for fluoride and chloride release. Abiotic and biological controls were included to quantify fungal contributions. Complementary bioinformatic analyses (Pfam/HMM domain mining, VCPO/CPO signature screening) were incorporated to identify putative oxidative, reductive, and vanadium-dependent enzyme systems linked to halogen metabolism.

Fungal cultures demonstrated clear transformation of both chlorinated and fluorinated substrates, with stoichiometric halide release confirming active dehalogenation. Toxicity assays indicated ~80% reduction in parent-compound toxicity and near-complete mineralization without detectable intermediates, contrasting with partial transformation typically reported for bacterial systems. Species-specific differences suggest concurrent oxidative and reductive mechanisms operating within fungal metabolism. These findings highlight a distinct and underutilized functional space for fungi in contaminant degradation.

The results provide a mechanistic foundation for integrating fungal biocatalysis into nature-based remediation systems, including engineered wetlands, fungal-amended biofilters, and organic-amendment matrices. Coupling fungal biochemistry with advanced analytical and genomic tools enables the development of scalable, low-impact treatment strategies aligned with sustainable environmental management.

Disclaimer/Disclosure:

Some genomic and enzyme-prediction data remain in preparation for publication and will be presented in summarized form.

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3.13.A.T-02 Two complementary ways to overcome bioavailability restrictions in bioremediation: direct enhanced desorption and solubilization

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The biodegradation of slowly desorbing polycyclic aromatic hydrocarbons (PAHs) in contaminated soils remains a major challenge in bioremediation. Biosurfactants offer a sustainable strategy to enhance pollutant bioavailability. While microbial biosurfactants like rhamnolipids are well-established, phytogenic surfactants such as saponin present a cost-effective, environmentally friendly alternative. Saponin is a non-ionic biosurfactant with excellent solubilization capacity for hydrophobic contaminants. This study investigates how saponin promotes PAH biodegradation through two complementary mechanisms: direct enhanced desorption and micellar solubilization. We employed a creosote-polluted soil containing 3,902 mg kg⁻¹ total PAHs. After 56 days of biostimulation, when most environmentally bioavailable PAHs were depleted, saponin was applied to mobilize the slow-desorbing fraction. Desorption kinetics of nine indicator PAHs were determined by Tenax extraction. Micellar solubilization was investigated in a NAPL/water biphasic system using ¹⁴C-labelled phenanthrene and pyrene at saponin concentration of 1 g L⁻¹ (above its critical micelle concentration of 0.1 g L⁻¹). Mineralization kinetics were correlated with PAH partitioning using the soil microbial community as inoculum. Results showed that direct enhanced desorption is the primary mechanism for enhanced biodegradation of slow-desorbing PAHs. Saponin sorption to soil aggregates swells the sorbent, enhancing intra-aggregate PAH diffusion and desorption. In biphasic systems, saponin induced contrasting effects: enhanced pollutant partitioning but inhibited microbial adhesion to the NAPL–water interface. Without saponin, phenanthrene mineralization rates exceeded partitioning rates. With saponin, mineralization kinetics shifted to linear rates corresponding to partitioning rates. We conclude that an initial biostimulation stage followed by saponin addition to degrade residual slow-desorbing PAHs is the most effective approach for improving soil bioremediation, and that balancing enhanced pollutant partitioning with inhibited microbial adhesion is critical for optimizing biosurfactant use in NAPL systems. These findings reinforce green chemistry approaches in bioremediation. Enhanced desorption by biosurfactant-containing biological fluids may increase PAH bioavailability in exposure scenarios such as earthworm gut and human respiratory tract, with implications for health effects.

3.13.A.T-03 Biological Granular Activated Carbon As A Reactive Barrier To Clean Up Groundwater Contaminated By PAHs.

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Background and Aim: Polycyclic aromatic hydrocarbons (PAHs) are persistent groundwater contaminants that demand long-term, sustainable remediation strategies. Permeable reactive barriers (PRBs) containing activated carbon (AC) are often used for PAH removal because of their strong adsorption capacity; however, this capacity is limited, leading to saturation, frequent media replacement, and reduced cost efficiency. To address the limitations, the present study investigates whether bioaugmented activated carbon (BAC), enriched with PAH-degrading microbial biofilms, can enhance PAH removal in PRBs by integrating adsorption with biodegradation. In BAC systems, saturated AC serves as a substrate for microbial colonisation, allowing microorganisms to utilise adsorbed organic matter as a nutrient source. As the biofilm matures across the carbon's pores and surfaces, it facilitates the biodegradation of both trapped and incoming contaminants. The goal of this study is to determine whether this self-cleaning mechanism can extend the operational lifespan and improve the overall performance of PRB systems used in groundwater remediation.

Methods: Batch experiments were conducted to compare pristine AC (adsorption only) and BAC (adsorption + biodegradation). A passive dosing approach using low-density polyethylene (LDPE) sheets preloaded with phenanthrene, pyrene, and perylene simulated gradual PAH release into groundwater. PAH concentrations were quantified using GC-MS following solvent extraction and nitrogen blowdown. The initial 28-day batch was followed by a 16-week extended experiment to observe long-term interactions.

Results: LDPE dosers released PAHs consistently, while pristine AC showed rapid initial adsorption approaching equilibrium after 42 days. In contrast, BAC maintained continuous pollutant removal beyond this point, suggesting biological removal, i.e. biodegradation. Among the tested compounds: Phenanthrene, pyrene and perylene, phenanthrene exhibited the highest biodegradation rate, achieving approximately 75% removal by day 6 in BAC systems.

Conclusion: BAC demonstrated superior and sustained removal of PAHs compared with pristine AC, confirming the potential for combined adsorption and biodegradation to occur in reactive barriers. The integration of BAC into PRBs offers a promising, sustainable solution for long-term groundwater remediation. Ongoing work involves column and microcosm studies to simulate realistic flow and PRB operating conditions.

3.13.A.T-04 Are there sustainable alternatives to biochar for reducing pollutant bioavailability? A look at biosealing bacteria

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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. This study evaluates whether *Bacillus subtilis* biosealing can serve as a sustainable alternative to traditional sorbents such as biochar, sand, and montmorillonite clay for reducing the bioavailability of hydrophobic organic pollutants. Naphthalene was used as a model contaminant to assess both adsorption and diffusion-limiting mechanisms. First, adsorption experiments in glass test bottles were conducted using equal masses of each material. Naphthalene concentrations were quantified by liquid scintillation, and Freundlich isotherms were constructed to compare sorption performance. All materials followed the Freundlich model with good agreement, confirming their capacity to immobilize naphthalene to varying degrees.

Second, diffusion through micrometre-scale pores was examined using membrane bioreactors equipped with 3 µm track-etched membranes. *B. subtilis* was applied on top of the membranes to induce pore clogging through biofilm formation. Naphthalene diffusing from the lower reservoir volatilized into the headspace and was trapped in cyclodextrin for daily quantification. The presence of *B. subtilis* markedly reduced naphthalene transport compared to bacteria-free controls, indicating effective biosealing of the pores. When combined with sorbents, diffusion decreased even further, with montmorillonite showing the strongest immobilization effect. Diffusion differences became evident after 2–3 days, consistent with the expected timeframe for bacterial deposition and pore clogging. Overall, the results demonstrate that *B. subtilis* biofilms can significantly limit contaminant mobility by physically obstructing pore spaces. When paired with clay or other sorbents, this strategy produces a synergistic reduction in naphthalene bioavailability, with sealing performance that surpasses conventional biochar in limiting naphthalene mobility through microporous media. These findings highlight biosealing as a promising, sustainable mechanism for mitigating pollutant spread in soils and porous media, potentially offering an alternative or complement to carbon-based amendments.

3.13.A.T-05 Metabolic pathways for biotransformation of benzalkonium compounds in fungal- and bacteria-based biofilm reactors

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Benzalkonium compounds are a biocidal disinfectant and can accumulate in sewage sludge and manure, where its persistence and transformation pathways remain poorly understood. This study addresses how different biological treatment systems degrade benzalkonium compounds and how microbial communities respond to exposure. To investigate this, biofilm reactors were operated in three setups: water, a fungal system using *Trametes versicolor*, and manure. Reactors were spiked with benzalkonium chloride and monitored for chemical transformation, metabolite formation, and microbial community shifts. Sampling was performed over time to assess degradation kinetics, transformation pathways and community adaptation through chromatography, high-resolution mass spectrometry and sequencing of ribosomal RNA genes. All systems degraded benzalkonium compounds but with distinct kinetics. Wastewater originating biofilms showed rapid removal through oxidation of the alkyl chain, followed by additional oxidation steps. The fungal system performed only partial oxidation, while the manure reactors exhibited the broadest range of metabolites, including benzylic transformations and dealkylation reactions. These differences reflect the influence of microbial diversity on degradation routes as microbial community analysis demonstrated clear differentiation between systems. In water, oxidative degradation was dominating. In manure, genera previously associated with transformation of quaternary ammonium compounds were enriched, coinciding with the appearance of manure-specific metabolites. Across all systems, benzalkonium chloride exposure did not consistently increase the abundance of the antibiotic resistance genes that were screened. Overall, this work demonstrates that biodegradation of benzalkonium chloride depends strongly on environmental matrix and microbial community composition, leading to distinct removal efficiencies and metabolite profiles. These findings improve understanding of disinfectant fate in biological treatment systems and support more effective management of quaternary ammonium compounds in wastewater and manure treatment.

3.13.B Harnessing Nature: Innovative Bioremediation and Phytotechnologies for Contaminant Removal and Ecosystem Recovery

3.13.B.T-01 VIRTUE: A Virtuous Cycle of Phytoremediation, Pyrolysis and Biochar Applications towards Safe PFAS Levels in Soil, Feed and Food

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Many agricultural, natural and urban areas are PFAS-contaminated from sewage sludge, fire-fighting foam and industry. This causes large economic and societal damages in many countries but remediation is challenging. The overall research need is a method for remediation for diffuse PFAS contamination without negative tradeoffs. We propose a Virtuous Cycle by combining phytoremediation to accumulate short-chain PFAS, then destroying them by pyrolytic treatment, and applying the resulting PFAS-free biochar as a sorbent to immobilize long-chain PFAS in soil and stormwater. We will present investigations of PFAS contamination of agricultural, urban and natural areas in Norway. We will also present data on uptake in plant species suitable for phytoremediation. We refined earlier work on pyrolysis conditions optimal for PFAS destruction and effective biochar sorbents and will show data on >99% reduction in PFOS leaching with 1.5-5% biochar made from local plants. The layout of a full LCA quantifying positive environmental impacts will be presented, as well as the principles of human exposure modelling and a Bayesian network approach to aid in optimal implementation scenarios. The data will be coupled to results from the ARAGORN project where remediation is tested for 9 contaminated sites across Europe, and both phytoremediation and biochar amendment are tested for PFAS and other types of chemicals (heavy metals, OCBs, PAHs). We aim to provide partners and society with a practical solution for the enormous problem of PFAS soil pollution, applicable to other contaminants.

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3.13.B.T-02 Biochar as Efficient Sorbents for the Retention of Road Runoff Pollutants in Tree Trench Biofilters

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Blue-green infrastructures, such as tree trench biofilters, have untapped potential to reduce subsurface contamination by road runoff pollutants. 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 for efficient transitory retention and slow pollutants release for subsequent biodegradation is still poorly understood.

Here, we compare untreated wood-derived biochar (BC) and gas-activated biochar (BAC) with respect to the retention of four typical road-runoff pollutants: naphthalene (NAPH), methyl tert-butyl ether (MTBE), 1,3-diphenylguanidine (DPG) and perfluorooctanoic acid (PFOA). Batch experiments were used to derive sorption isotherms and sorption kinetics; Flow-through retention was evaluated in small columns and compared with FAST 2.0 and AdDesignS predictions based on batch-derived isotherm and diffusion parameters; Pilot-scale tests were conducted in a 1 m³ IBC unit subdivided into four compartments with identical substrate and gravel layers, but different top layers (soil, soil with grass, soil with BC, soil with BAC).

From the batch experiments, all pollutants showed substantially higher adsorption on BAC than on BC, with more than an order of magnitude increase in capacity despite an only 2.4-fold increase of its surface area. Kinetic data revealed strong diffusion limitations in BC, while mesopores in BAC enabled markedly faster intraparticle transport. Column experiments confirmed rapid initial DPG removal by BC followed by early breakthrough (about 40 pore volumes), whereas model simulations predicted more than 70% sustained removal for BAC over 500 pore volumes. Pilot-scale results corroborated these trends: BAC achieved the highest pollutants retention, whereas BC performed similarly to the grass and soil treatment. Overall, BAC proved to be the most suitable amendment for tree trench biofilters, as confirmed across batch tests, column experiments, modelling, and pilot-scale test systems. This integrated approach provides a robust framework for scenario-based design of biochar-amended biofilter systems.

3.13.B.T-03 Comparative Study of Nature-Based Reclamation of Distinct Greywater Streams

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Water reuse is increasingly prioritised in the Mediterranean region, despite technical, social and safety barriers. Nature-based solutions, e.g. green walls, offer environmentally friendly options for water reuse, nutrient recovery and urban agriculture. However, their performance is still insufficiently documented, particularly for greywaters. This study aimed at evaluating: (i) the treatment efficiency of two full-scale demonstration green wall facilities operating with real greywater, and (ii) the suitability of their effluents for reuse. The first system, GRETA (Sant Quirze del Vallès, Spain), has operated since April 2023 treating up to 220 L·day⁻¹ of shower, sink and rainwater using 8 planted modules (substrate perlite, gravel, LECA with 24 plant species), horizontal flow and an ozonation step. The second facility, the experimental green wall (EGF) at ICRA (Girona, Spain), has operated since January 2025 treating 180 L·day⁻¹ of kitchen and sink greywater with four modular sectors combining vertical and horizontal flow, LECA–perlite substrates and 5 plant species. Water quality was monitored for 3-4 weeks at both systems, analysing regulated parameters and contaminants of emerging concern. Samples were extracted by SPE and analysed by LC-MS/MS (PPCPs, EDCs, plastic additives) and LC-HRMS (suspect screening). Environmental safety was assessed through risk quotients and current regulations. Both systems showed good physicochemical and microbiological performance. GRETA achieved high removals of ammonia (99%), total nitrogen (80%), E. coli (>99%), organic matter (80-98%) and TSS (96%). EGF removed 80–90% of organic matter without increasing alkalinity or TSS. GRETA showed high removal of several pharmaceuticals (ibuprofen, acetaminophen 99–100%; valsartan 88%; fluoxetine 100%), while others improved after ozonation (carbamazepine 20–93%). Caffeine was frequently detected yet efficiently removed (94%). Some antibiotics showed negative removal in the first step but were eliminated by ozonation. Chemical analyses and risk assessment for EGF are ongoing. Overall, green wall systems

demonstrate strong potential for treating greywater in urban buildings, meeting reuse standards and effectively removing organic micropollutants. These results support the development of innovative, decentralized and cost-effective water reuse schemes that contribute to water-scarcity resilience and sustainable circular water systems.

Disclaimer/Disclosure:

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3.13.B.T-04 Remediation of PFAS Contaminated Soil Using Sorbent Stabilization with Activated Carbon and Sewage Sludge Biochar: Insights on Plant and Earthworm Uptake and Ecotoxicity

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Two potential effective (bio)char types for remediation of soils contaminated with per- and polyfluoroalkyl substances (PFAS) are activated carbon (AC) [2] and sewage sludge-based biochar (BC-SS) [3]. Both have demonstrated similar, large sorption capabilities for longer chain PFAS, however, sorption of shorter chain PFAS may be less effective and only few studies have investigated sorbent effects on zwitterionic PFAS. Furthermore, the profound effects that these sorbents can have on uptake by plants and earthworms and potential ecotoxic effects also remain an area requiring further research.

Two PFAS-contaminated soils (“Med PFAS” 750 µg ΣPFAS kg⁻¹ soil; “High PFAS” 2000 µg ΣPFAS kg⁻¹ soil) were collected from former firefighting areas in Sweden. The soils were treated either without any additions (Control); 1% (w/w) compost (+ fertilizer in plant test) (FC); 1% compost and 2% of a wood-based commercially available AC (BC-AC), or 1% compost and 2% BC-SS (BC-SS). The mixtures were tested for PFAS solubility using a batch leaching test, plant growth and PFAS uptake in perennial ryegrass *Lolium perenne*, and acute toxicity, reproduction and PFAS uptake in earthworms *Eisenia fetida*. Up to 54 neutral, anionic, cationic and zwitterionic PFAS were targeted and analyzed using UPLC MS/MS.

Both biochars resulted in very effective in stabilization of PFAS in the soils. In the Med PFAS soil, ΣPFAS decreased with 95.7% in the leachate with BC-SS and 99.6% with BC-AC, with compost contributing to the retention. Plant growth increased with compost, biochar and fertilizer addition, with the highest biomass observed using BC-SS. Uptake of PFAS in earthworms and plants was highly reduced in BC-AC treatments (resp. 97% and >99% reduction of ΣPFAS). BC-SS treatments showed similar reduction of PFAS uptake in earthworm (95%) but some remaining short-chain PFAS uptake in plants (75% reduction of ΣPFAS). However, BC-AC and in a lesser extent also BC-SS decreased earthworm biomass and reproduction. In conclusion, we show that these biochars are effective PFAS stabilizers and provide a promising approach to stabilize PFAS in situ. However, there is a need to balance sorbent addition with potential negative side-effects to biota.

3.13.B.T-05 Pilot Scale Study for a Green Recovery of a Construction Site Area

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Tunnelling projects can significantly affect natural ecosystems, causing habitat loss and degradation as result of extensive surface soil removal for setting up a construction site. The soil is piled up for long time up, with a consequent loss of vegetation cover at the construction site. Moreover, millions of cubic metres of soil excavated (spoil material, SM) are generated for tunnel implementation. Although SM can be valorised and reused (in accordance with circular economy principles), its chemical stabilization with lime (LSM) for facilitating its handling, owing to high pH values, can influence its use for greening purposes.

This work reports a case study related to the A1 highway tunnel construction (Apennine Mountains near Florence, Italy), where a revegetation planning is underway. Four-year mesocosms consisting of mixed SM with surface soil and different organic amendments (compost, pomace and digestate) tested the growth of five native tree/shrub species: *Ostrya carpinifolia* (black hornbeam), *Quercus pubescens* (downy oak), *Fraxinus ornus* (ash),

Olea europaea (olive) and *Corylus avellana* (hazel). The mesocosms consisted of a topsoil layer (0–30 cm depth, initial pH: 8), an intermediate layer (35–50 cm depth) consisting of LSM amended with compost or pomace or digestate and a deeper LSM layer (50–60 cm, depth pH: 11–12). Two control conditions (only topsoil and untreated SM) were also set-up. Plant survival and growth, soil parameters (e.g. pH and organic carbon) and microbial community abundance and activity were evaluated over time. Organic amendments improved soil quality, thanks to the introduction of active microbial populations if compared with controls. At 48 months, pH values were comparable both in the amended and non-amended conditions, indicating that microorganisms played a key role in moderating the alkaline conditions of SM and facilitating its mitigation. In fact, microbial taxa, which have in their functional profile genes involved in pH homeostasis, have been identified. Moreover, tree mortality was quite low (15%) and mostly affected oaks (*Quercus pubescens*, 38%). Tree performances among different amendments did not show statistical differences, except one species (*Olea europaea*) grown on surface soil (control) which had the best performance because the pH was lower than 8 in all layers.

3.13.P Harnessing Nature: Innovative Bioremediation and Phytotechnologies for Contaminant Removal and Ecosystem Recovery

3.13.P-We243 Assessment via Target and Suspect Screening of the Fate of Mobile Organic Contaminants and Transformation Products in Sustainable Urban Drainage Systems

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Rapid urbanization and climate change are driving a transition from grey infrastructure to nature-based solutions like Sustainable Urban Drainage Systems (SUDS) for stormwater management. While effective for filtering conventional pollutants (e.g., metals, solids), their efficacy against mobile organic contaminants remains elusive. In particular, due to high water solubility and low sorption, polar Persistent, Mobile, and Toxic (PMT) substances often bypass biofiltration. Furthermore, biological activity within SUDS may facilitate incomplete degradation, generating Transformation Products (TPs) that could be more persistent or toxic than their precursors and are not included in standard monitoring.

This study assesses the fate of mobile contaminants across the urban infiltration pathway in Barcelona. The objectives were to: i) develop a targeted HPLC-QqQ method for 52 contaminants (pesticides, industrial chemicals, tyre-related compounds, and PPCPs); and ii) analyze known and in-silico predicted TPs via HRMS suspect screening. Sampling campaigns covered first-flush stormwater, SUDS effluents, and groundwater. A specialized sample preparation protocol using lyophilization and reconstitution was validated to overcome SPE limitations for hydrophilic substances, yielding recoveries of 70–120%.

Analysis revealed a high occurrence of polar anthropogenic contaminants in street runoff, with concentrations reaching 10 µg/L. Comparing influents with effluents showed that while SUDS significantly reduce the total contaminant load, specific polar compounds exhibit "breakthrough" behavior. Notably, hazardous PMTs such as TCPP and Melamine were found enriched or poorly retained in effluents. Additionally, suspect screening will tentatively identify TPs formed during infiltration.

These findings indicate that SUDS, while promising, may act as source of mobile chemicals into the underneath zone. This underscores the necessity of integrating advanced monitoring to evaluate the chemical security of green infrastructure and protect groundwater resources.

Disclaimer/Disclosure:

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3.13.P-We244 Links Between Environmental Heterogeneity, Microbial Community Assembly, and Micropollutant Removal in Constructed Wetlands

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Constructed wetlands (CWs) are widely used as nature-based solutions to improve water quality. In urban areas, they often receive stormwater or treated wastewater discharged directly from wastewater treatment plants. CWs receiving treated effluents play a crucial role in intercepting micropollutants, such as pharmaceuticals, personal care products, and pesticides. Despite their importance, the mechanisms through which CW design, vegetation structure, and habitat heterogeneity shape microbial communities and facilitate micropollutant removal remain poorly understood. This knowledge gap limits our ability to optimize CW performance for eliminating organic contaminants that persist after conventional wastewater treatment. Although CWs are less heterogeneous than natural lakes or rivers, they contain substantial small-scale variability. Differences in ponds shape, pollutant loads, plant species, and vegetation cover create environmental gradients that generate diverse microhabitats. These microhabitats can influence microbial community assembly and, in turn, affect the capacity of CWs to attenuate organic micropollutants. We therefore hypothesize that CWs with greater within-system heterogeneity will support more taxonomically and functionally diverse microbial communities, resulting in enhanced micropollutant biodegradation. Previous research has largely relied on laboratory studies to evaluate microbial and micropollutant dynamics, but these controlled settings rarely capture the shifting environmental conditions and complex chemical mixtures present in full-scale systems. As a result, our understanding of how full-scale CWs function at the microbial level remains incomplete. This study aims to investigate how environmental heterogeneity across and within CWs influences microbial community composition and micropollutant attenuation. Fieldwork was conducted between June and October 2025. During this period, water and sediment samples were collected from nine horizontal surface-flow CWs in Sweden to profile microbial communities and quantify organic micropollutants. Additionally, plant composition and vegetation cover were assessed through field surveys, environmental DNA analysis, and aerial imagery. The results will highlight how engineered ecosystems can be designed or managed to maximize micropollutant removal and support the development of more effective and ecologically grounded wastewater treatment.

3.13.P-We245 From pilot to deployment: a high-efficiency, nature-based technology for contaminant removal and ecosystem recovery

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Water pollution is a defining environmental and public health challenge. Conventional wastewater treatment plants (WWTPs) were never designed to remove persistent, mobile, and toxic (PMT) chemicals, such as pharmaceuticals, pesticides, microplastics, and PFAS. These chemicals, some known as “forever chemicals”, pass largely untreated through current systems, accumulate in surface and groundwater, and eventually return to our table through the food chain. Their growing presence threatens drinking water security, degrades ecosystems, increases human health risks, and raises regulatory pressures on utilities and industries.

Daphne Water Solutions (DWS) Ltd. addresses this gap with a patented, nature-based tertiary treatment technology developed over more than a decade of research. The system harnesses the biofiltration capacity of *Daphnia* (water fleas), freshwater organisms long used as sentinel species in ecotoxicology. Unlike many nature-based approaches, which are slow and infrastructure-intensive, the DWS system is engineered for speed and scalability. It operates within the hydraulic retention times of municipal WWTPs, removing contaminants in hours rather than days. It is modular, retrofittable, and produces no toxic by-products.

Here, we report results from near real-world demonstrations, including a 12-month continuous operation of a 2.7 m³ prototype treating secondary effluent. The system achieved removal efficiencies of up to >93% across 32 target contaminants, including PFAS and microplastics. Based on these outcomes, DWS has advanced to commercial-scale pilots at large WWTPs and decentralized deployments in rural and heritage sites, testing the system under diverse operational and regulatory conditions.

A recent life cycle assessment (LCA) further quantified the environmental benefits of the technology, showing a 20% reduction in freshwater ecotoxicity potential and an 8% reduction in eutrophication risk compared to conventional and nature-based tertiary treatments. These improvements make the DWS technology a game changer for enhancing water quality while actively protecting biodiversity and human health.

The platform offers a scalable, energy-efficient, and low-impact solution that aligns with circular water strategies and accelerates progress toward Net Zero environmental goals.

Disclaimer/Disclosure:

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3.13.P-We246 Lignosulphonate, A Green Surfactant-Driven Bioavailability to Accelerate Bioremediation: A Circular Strategy to Address Recalcitrant Contaminants in Polluted Soils and Sediments

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Bioremediation-oriented clean-up technologies offer a viable solution for the removal of persistent organic pollutants (POPs) and reclamation of polluted soil and sediments. However, the limited bioavailability of POPs within the complex environmental matrices remains a key bottleneck in achieving successful bioremediation. Enhancing bioavailability substantially improves microbial access to recalcitrant pollutants, thereby accelerating biodegradation and resulting in effective clean-up. Recently, surfactant-aided strategies have shown promise in enhancing the bioavailability of hydrophobic organic contaminants, aiding microbial metabolic breakdown and accelerating the rate of bioremediation. Despite their advantageous role, the environmental drawbacks of chemical surfactants and the expensive manufacturing cost associated with biosurfactants demand the need for viable, sustainable alternatives. In this context, the present study focuses on investigating the potential of lignosulfonate, a sulphite pulping by-product comprised of lignin and sulphonate functional groups that confer surface-active characteristics, as an environmentally friendly surfactant for enhancing the pollutant bioavailability during bioremediation. In addition, its established application in soil stabilisation signifies its suitability as a potential safer candidate for environmental applications, while its origin as an industrial side stream contributes to resource circularity. In this study, the impact of lignosulphonate surfactant on the bioavailability of a broad spectrum of emerging contaminants, including Polychlorinated biphenyls, polycyclic aromatic hydrocarbons, dioxins and related hydrophobic pollutants present in aged, contaminated soil and fibre-rich sediments will be evaluated. Then, the lignosulphonate performance will be systematically compared with different classes of other green surfactants—such as rhamnolipid, Eco-Tween 80, and saponin. The surfactant-mediated enhancement in contaminant bioavailability will be further benchmarked against the environmental bioavailability of POPs determined by the standardized desorption extraction method using TENAX, which is a valuable tool used in environmental risk assessment. The study is anticipated to explore how lignosulphonate can act as a safe, low-cost green surfactant that improves bioremediation efficiency, thereby advancing the successful mitigation of hazardous pollutants and the restoration of contaminated environments.

Disclaimer/Disclosure:

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3.13.P-We247 Removal of per- and polyfluoroalkyl substances in a Multifunctional Decentralized Wastewater Treatment System

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Water scarcity and the need for resilient water management are driving interest in local wastewater reuse. Per- and polyfluoroalkyl substances (PFAS), particularly ultra-short-chain compounds such as trifluoroacetic acid, persist through conventional treatment and pose environmental and human health risks. This study evaluates a pilot-scale, multifunctional, decentralized system that combines an aerated vertical-flow constructed wetland with an activated carbon filter derived from sewage sludge, with the aim of enhancing PFAS removal.

The treatment train for municipal wastewater treatment at the BDZ site in Leipzig included primary sedimentation in a three-chambered septic tank, an aerated vertical-flow constructed wetland (supporting plant-microbe interactions) for secondary treatment, and additional tertiary treatment steps (optional): slow sand filtration, ultraviolet irradiation, and activated carbon filtration. Composite samples were collected over five weeks across the three treatment steps. PFAS, from ultra-short to long-chain compounds including precursors, were quantified using targeted liquid chromatography-mass spectrometry.

The biological treatment in the constructed wetland only achieved limited PFAS removal, with some increases potentially due to precursor transformation. Tertiary treatment steps incrementally improved removal performance, while activated carbon achieved the highest removal for short and long-chain PFAS. Certain ultra-short PFAS showed partial reduction, whereas trifluoroacetic acid and trifluoromethanesulfonic acid remained persistent. Overall, the integrated system outperformed conventional centralized wastewater treatment plants for removing most of the PFAS.

These findings demonstrate that decentralized, multifunctional systems integrating engineered natural processes with waste-derived activated carbon can enhance PFAS elimination, support safer wastewater reuse (e.g., agricultural irrigation purposes), and offer a scalable, low-impact alternative to conventional treatment. Ongoing work also investigates plant uptake and optimization to address persistent ultra-short PFAS, highlighting the potential of nature-based strategies for sustainable contaminant management and ecosystem protection.

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3.13.P-We248 Two stage biological removal of chlorophenoxy acid pesticides combining moving bed biofilm and biofiltration reactors from contaminated groundwater. A pilot-scale study

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Across Europe, groundwater is increasingly affected by complex mixtures of legacy and emerging contaminants, including pesticides and their transformation products from production residues. Within this context, nature-based and low-impact remediation systems provide promising alternatives to conventional treatments. A pilot study was conducted at the Stengården landfill, which was loaded with municipal wastes as well as production residues of pesticides site in Denmark to evaluate a biologically driven treatment process for pesticide-contaminated groundwater under high iron concentrations.

The pilot system combines Moving Bed Biofilm Reactors (MBBR) and biofilters, representing an engineered natural system designed to enhance microbial degradation while minimizing energy and chemical inputs. The study aimed to quantify removal efficiencies for the herbicides mecoprop, dichlorprop and their metabolites, as well as to investigate how environmental and process factors such as iron, hydraulic retention time, dissolved oxygen and temperature influence biotransformation pathways. Monitoring included targeted chemical analyses and operational parameters to assess performance under realistic field conditions.

The system achieved high removal of the parent pesticides (85 percent on average) and moderate removal of the metabolites (60 percent). Effluent concentrations of mecoprop and dichlorprop consistently met the European Union drinking water limit of 0.1 µg/L, while metabolite concentrations remained above the threshold. Removal efficiencies showed positive correlations with dissolved oxygen in the biofilter. Elevated iron levels, approximately 30 mg/L, inhibited mecoprop degradation, suggesting an interaction between redox dynamics, microbial activity and contaminant transformation.

This work demonstrates how engineered biological systems can support the treatment of groundwater impacted by emerging contaminants, offering a scalable and energy-efficient nature-based solution. The findings improve understanding of pesticides from production residues degradation under variable environmental conditions and contribute to the development of sustainable remediation strategies aligned with the principles of the European Green Deal and ecosystem restoration initiatives.

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3.13.P-We249 Plastic additive enrichment drives shifts in microbial communities with traits of plastic degradation

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Plastics incorporate numerous chemical additives that improve material performance but also contribute to environmental contamination due to their persistence and tendency to leach. Microbial degradation provides a promising route to mitigate these pollutants, though effective screening approaches for active degraders remain limited. This study combined biochemical assays with 16S rRNA amplicon sequencing to identify microbial communities capable of degrading common plastic additives. Microbial inocula from contrasting environments – natural (Chessel Bay, Swansea Bay) and anthropogenic (wastewater treatment and recycling plants) – were enriched for 22 days using di(2-ethylhexyl) phthalate (DEHT), tetradecane, or a no-additive control. High-throughput assays were adapted to quantify community-level growth, cell death, redox potential, and esterase activity across treatments. DEHT cultures supported the greatest microbial growth, while tetradecane exposures showed enhanced redox activity, suggesting distinct metabolic responses to ester versus alkane additives. Sequencing revealed 957 amplicon sequence variants across 36 cultures, with PERMANOVA indicating that substrate type explained 39–63% of the variance in community composition. Both additives enriched bacterial taxa with recognized plastic-degrading potential, such as Nocardiaceae, which correlated with elevated esterase activity. Intriguingly, families not previously linked to plastic degradation, including Vermiphiliaceae, also increased in relative abundance, suggesting untapped biodegradative capacities within environmental microbiomes. Overall, these results demonstrate that diverse microbial communities from natural and engineered systems can metabolize ester- and alkane-based plastic additives. The integrated biochemical–molecular approach developed here enables scalable identification of biodegradative consortia and holds promise for advancing microbial bioremediation of plastic-associated pollutants.

3.13.P-We250 Role of plant leaves in the degradation of airborne pollutants: biodegradation vs. photodegradation of Polycyclic Aromatic Hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic contaminants, that can derive from the incomplete combustion of wood and fossil fuels and vehicular traffic. Once emitted they can be found in the atmosphere in gaseous form or associated to particulate matter. Plants can remove PAHs from the air through the so called Forest Filter Effect (FFE) accumulating these compounds in leaves and transferring them to soil. Moreover, the phyllosphere host significant amounts of bacteria that can contribute to the biodegradation of PAHs producing metabolites such as OH-PAHs. However, no studies are available so far about the presence and source of PAH metabolites in plant leaves. Just few works reported data about their photodegradation on leaf surfaces.

In this work, new aspects of FFE were investigated through several laboratory experiments of increasing ecological complexity. More specifically, the study was designed to evaluate for the first time the contribution of light and phyllosphere microbial communities of *Quercus ilex* leaves to the degradation of deuterated PAHs (naphthalene-d₈, acenaphthene-d₁₀, phenanthrene-d₁₀, pyrene-d₁₀, chrysene-d₁₂, and perylene-d₁₂). Such degradation was investigated by observing the production of deuterated PAH hydroxy metabolites (OH-PAHs). This was performed through target and suspect screening analyses of PAH metabolites using an UHPLC-HRMS (LC-Orbitrap).

OH-PAHs were produced both in light and dark conditions (generally after 7 days), while their appearance in sterile controls was negligible. The number of PAH metabolites and their isomers increased when entire leaves were used rather than the inoculated phyllosphere microorganisms only, confirming the degradation ability of native phyllosphere microbial communities. Although preliminary, these results showed that FFE can be enhanced by photo- and biodegradation processes, because these degradation reactions could help to maintain a high air to leaf PAH gradient, since these compounds could be continuously removed. Additionally, the metabolites produced, being more polar and less hydrophobic, may likely be washed off during rainfall events, and be transported to the terrestrial environment, restoring the PAH gradient.

Further and more realistic studies are necessary to better quantify the contribution of phyllosphere biodegradation and photodegradation on PAH removal from air (phylloremediation) and their precise role in FFE.

3.13.P-We251 Iron-Enriched Biochar for the Removal of Mn(II) and Ni(II) from Drinking Water

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Highly-contaminated drinking water with Mn(II) and Ni(II) poses public health risks, including adverse neurodevelopmental effects and eczematous flare-ups in children. Efficient removal of these Potentially Toxic Elements (PTEs) is crucial to ensure safe drinking water consumption, as described by the UN Sustainable Development Goal 6: Clean Water and Sanitation. Iron-based materials have emerged as low-cost, effective, and clean alternatives to activated carbon for water treatment; however, their application is limited by aggregation, surface passivation, and low selectivity. Biochar has increasingly been used as a carrier material to mitigate these limitations due to its high specific surface area, functional groups, and conductivity. However, no studies have evaluated the efficiency of iron-enriched biochar for Mn(II) and Ni(II) removal from synthetic aqueous solutions or real drinking water. The aim of this study is to assess the efficiency of iron-enriched biochar for Mn(II) and Ni(II) removal from synthetic solutions and highly-contaminated drinking water from Longyearbyen, Svalbard. Iron-enriched biochars will be produced by co-pyrolysis of Brewer's Spent Grains and hematite (Fe₂O₃) at 400, 500, 600, and 700°C, and tested in batch experiments using synthetic solutions and contaminated drinking water samples. Adsorption experiments will be conducted under various conditions (temperature, pH, contact time, and sorbent dosage) to determine removal efficiencies, kinetics, and isotherm behavior. Iron-enriched biochar characterization (elemental analysis, FTIR, XRD, SEM-EDS, etc.) will be exploited to link material properties, iron species formation, and surface chemistry to observed removal mechanisms. We expect that increasing pyrolysis temperature will enhance biochar conductivity; thus increasing electron-transfer rates for removal of Mn(II) and Ni(II). However, higher temperatures are also expected to decrease the abundance of surface functional groups; thus reducing metal-ion complexation. The overall removal efficiency will therefore depend on the balance between these two temperature-dependent effects. The results of this study will provide insight into the suitability of iron-enriched biochar as a low-cost, environmental-friendly technology for Mn(II) and Ni(II) removal from real drinking water, and contribute to future work on urban farming in the Arctic and its potential application to soils to limit plant uptake of PTEs and heavy metals.

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3.13.P-We252 Uptake and Depuration of Short- and Long- Chain Per- and Polyfluoroalkyl Substances in Submerged, Floating and Emergent Aquatic Plants

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Per- and polyfluoroalkyl substances (PFAS) are persistent anthropogenic chemicals, widely used in industrial and consumer products for their water and grease-resistant properties. They are major pollutants of water bodies and, due to their persistence and bioaccumulation, they pose a danger to aquatic organisms. Aquatic plants can remove PFAS from contaminated water through uptake and potential enzymatic degradation. Different wetland species vary in their PFAS uptake capacity, influenced by plant type and biomass per water volume. PFAS can enter plants through roots or the entire plant structure in submerged species, floating plants are in contact with the upconcentrated levels at the air-water boundary, and emerging plants translocate PFAS out of the water into their leafy and reproductive tissues. Aquatic plants are a potential, cost-effective solution for the remediation of polluted water bodies and a nature-based alternative to costly conventional treatment methods. For this purpose, three types of plants were tested with two PFAS, through an uptake and elimination phase, one submerged plant (*Elodea canadensis*), one floating (*Salvinia natans*) and one emerging (*Glyceria maxima*). To evaluate the role of chain length in uptake, one long-chain (PFUnDA) and one short-chain (TFA) PFAS compound were utilised in two concentrations (50 µg/L and 1000 µg/L). In the uptake phase (lasting 14 days) the plants were exposed to the PFAS, while in the depuration phase (14 days) the plants were transferred to clean medium, to assess pollutant uptake and release. Sampling was performed on days 0, 1, 4, 11, 14, 15, 20, and 28 and the internal concentrations were measured in different plant compartments (e.g., roots and shoots). Additionally, for the emergent plant, the shoots were divided into the parts below and above the water. The internal concentration results from each plant compartment were further analysed using a toxicokinetic model. This study aims to advance understanding of how aquatic plants can mitigate PFAS pollution by examining species-specific

differences in uptake, translocation, and depuration of short- and long-chain PFAS. The comparison of submerged, floating and emerging species can clarify the role of plant morphology and exposure pathways of each specific compound on PFAS removal efficiency. The findings will contribute to the development of nature-based, low-cost phytoremediation strategies for PFAS pollutants in aquatic environments.

3.13.P-We253 Degradation and formation of PACs during the bioremediation of creosote-polluted soil combined with toxicity and microbial community analysis

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PAH-contaminated sites typically contain highly complex contaminant mixtures that include not only PAHs but also a range of other substituted polyaromatic compounds (PACs), such as alkylated-PAHs, NSO-heterocyclic PACs and oxygenated PAH transformation products (oxy-PAHs). Oxy-PAHs may also form during bioremediation treatments and are known to potentially increase soil genotoxicity despite effective PAH removal. A central challenge in the bioremediation of PAH-contaminated soils is to elucidate the actual in situ processes by analyzing microbial communities as functional systems and broadening risk assessment beyond the traditional 16 US-EPA priority PAHs. In this work, we evaluate how microbial biostimulation influences the fate of more than 80 analytical targets and soil toxicological endpoints, and we identify the microbial processes that drive the formation and eventual subsequent degradation of toxic transformation products.

Soil slurry microcosms were set up using a heavily creosote-polluted soil and incubated for 50 days under different biostimulation regimes (with or without nutrient addition). Soil extracts were obtained using a Dionex ASE 350 with in-cell basic silica clean-up. To recover a wider range of polar transformation products, aqueous samples were extracted using a combined neutral and acidic SPE extraction workflow developed in this study. Soil and neutral aqueous extracts were analyzed by GC-LRMS, whereas acidic extracts were analyzed by LC-HRMS. Toxicological risks were evaluated through cell line-based CALUX® bioassays. Microbial community dynamics involved in PAC degradation were examined by tracking structural changes at both genomic and transcriptomic levels using 16S rRNA gene sequencing.

The native soil microbial communities responded to bioestimulation by removing more than 80% of PAHs when nutrients were supplied. In the absence of nutrients, degradation of high-molecular-weight PAHs (HMW-PAHs) was limited, and these compounds tended to leach into the aqueous phase. Notably, an increase in toxicity was observed after 20 days, coinciding with maximum PAC biodegradation rates. This suggests that transformation products, not included in our extensive analytical target list, contributed to the increase in toxicity. Total and active microbial communities exhibited clearly distinctive compositions, and nutrient addition modulated the succession of active taxa associated with PAC biodegradation.

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3.13.P-We254 Organic Micropollutant Transformation Products in *Brachypodium distachyon*

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Organic micropollutants (OMPs) are a global environmental concern posing a threat to water quality. Plants are intentionally or unintentionally exposed to OMPs during water purification purposes (constructed wetlands) or food production (pesticide application; irrigation with treated effluent), respectively. Although the potential of plants to biotransform xenobiotic chemicals is known, there is little knowledge of the chemical identity of actual in-plant transformation products (TPs). Many aspects play a role when it comes to OMP TPs in plants, starting with the bioavailability of OMPs (i.e. not sorbed to soil), plant uptake behavior and plant defense mechanisms. The low concentrations at which OMPs occur in the environment, the potentially lower concentration of TPs in plants, and the complex plant matrix make the identification and quantification of OMP TPs in plant material challenging. To evaluate the fate of OMPs in plants and, more specifically, their spatial distribution within

plants, it is crucial first to identify TPs. The study aims to provide insight into the fate and transformation of a variety of OMPs with different chemical properties after their uptake in the plant. It was hypothesized that plant uptake is OMP specific. In case of uptake, not all OMPs will be transformed. If TPs are formed, we expect them to undergo a similar transformation, including hydrolysis, oxidation, and glycosylation. To identify TPs, we exposed model plant species *Brachypodium distachyon* to different OMPs in a hydroponic growth set-up. Targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was performed to quantify OMP uptake into roots and potential transport into the shoots. In addition, non-targeted LC-HRMS analysis on shoot and root material were performed to provide insights into TPs formed in-plant, and to reveal changes in overall plant metabolite composition and hence plant wellbeing. The data processing tools GNPS and patRoom were used for data analysis. Valuable insights are gained from the explorative approach, adding experimental data to OMP in-plant alteration model. In summary, with this poster presentation, I will present an experimental design that allows for the investigation of plant OMP uptake, transportation and transformation. This knowledge allows further investigation into in-plant mode of action or risk assessment of OMPs in the environment.

3.13.P-We255 Harnessing Recycled Manganese/Iron Oxide Sand In Constructed Wetlands For Enhanced Organic Micropollutant Removal

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Managed aquifer recharge (MAR) with surface water in the Netherlands is hampered by the presence of organic micropollutants (OMP) in this surface water. As a result, Dutch drinking water companies are testing constructed wetlands as an innovative pretreatment strategy before MAR. While conventional constructed wetland designs effectively remove COD and nutrients, they fail to sufficiently remove all OMP.

To overcome this, we propose integrating manganese/iron oxide (MnOx/FeOx) sand recycled from drinking water treatment plants as the substrate in constructed wetlands. This recycled MnOx/FeOx sand is biologically generated as a byproduct of microbial oxidation of dissolved Mn²⁺ and Fe²⁺ in groundwater-based drinking water production plants.

These biogenic oxides offer three potential OMP removal mechanisms: 1) Chemical Oxidation: Electron transfer from OMP to the highly reactive MnOx matrix. 2) Biodegradation: Degradation and MnOx regeneration mediated by manganese-oxidizing bacteria (MnOB) embedded in the biofilms. 3) High adsorption capacity: Biogenic MnOx possesses a δ -MnO₂ crystalline structure to enhance OMP adsorption.

To validate the concept, batch OMP removal experiments will be performed with 24 OMPs, including Glyphosate, Sulfamethoxazole, Diclofenac, Sucralose, etc. Controlled mixtures of recycled MnOx/FeOx sand and mature sand from running constructed wetlands will be used as an inoculum and tested under 1) Inhibited Systems (4°C): Isolating abiotic processes (adsorption + oxidation, 2) Biotic Systems (20°C): Enabling all three mechanisms. The OMP removal efficiency will be tested under both strict aerobic and anaerobic environments for 28 days and monitored using LC-MS/MS. In addition, high-resolution LC-MS will be used for non-target screening to identify transformation products.

By performing these batch experiments, we hypothesize that we will be able to determine the contribution of MnOx/FeOx sand to the overall OMP removal efficiency and identify the respective contributions of biodegradation, adsorption, and chemical oxidation.

The findings of these batch experiments aim to provide valuable insight into the critical parameters that significantly impact the interaction of MnOx with OMP removal, ultimately affecting OMP removal efficiency. The result will be consecutively used in the design of lab-scale and pilot-scale MnOx-mediated wetland construction, such as the oxygen level (water saturation zone) and hydraulic retention time, etc.

3.13.P-We256 Plant-microbe interplay influencing urban pollutant transformation in percolation systems

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Urban runoff transports diverse organic pollutants that threaten urban waters and soils. Blue-green infrastructures such as tree trenches help to mitigate these impacts. Yet, the biological contribution of the rhizosphere to pollutant transformation remains understudied. We ask whether plant-microbe interactions and low molecular weight carbon amendments can stimulate pollutant transformation under realistic conditions. We hypothesize that low molecular weight carbon can stimulate microbial activity and support co-metabolic

degradation. To test this hypothesis, we established an outdoor mesocosm experiment. Trees (*Tilia cordata*) were planted in 1000 L containers that simulate a real tree trench in Leipzig, Germany. The systems received a mix of pollutants commonly found in urban runoff waters (naphthalene, bis(2-ethylhexyl) phthalate, methyl tert-butyl ether, and 1,3-diphenylguanidine). We added by-products from industries (molasses, whey) as low molecular weight carbon to stimulate microbial processes. We also applied four surface cover treatments: biochar, activated biochar, grasses, and an uncovered control. We monitored key biogeochemical and biological indicators. These include pH, redox potential, nitrogen and carbon species, 16S rRNA genes and transcripts, and functional genes involved in pollutant degradation. This approach allows us to assess both general microbial responses and the activation of specific degradation pathways. The chemical fate of the added pollutants is also being quantified. The analytical work has only recently begun and laboratory processing is still ongoing. Early observations show that organic priming increases microbial activity. First signals also indicate shifts in carbon cycling. These responses suggest that priming can enhance conditions that support co-metabolic degradation. Full chemical and molecular analyses will confirm whether these biological changes translate into higher transformation rates.

This work provides new insights into how biological mechanisms influence contaminant fate in tree trenches. Understanding these interactions supports the design of blue-green infrastructures that not only retain pollutants but also promote their transformation. This contributes to more resilient and sustainable urban water management.

3.13.P-We257 A trend prediction model of natural attenuation in groundwater based on machine learning and microbial community

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Natural attenuation combined with long-term monitoring (known as monitoring natural attenuation, MNA) has been proven to be a resultful remediation technology in petroleum-contaminated sites in which the biodegradation was the main study conducted at contaminant removal. Trends analysis and prediction are important segments for MNA application, in which complex biotic and abiotic factors need to be considered. Microorganisms also could be regarded as “first responders” to environmental changes, and carries additional information about environmental conditions that should be of great advantage in simplifying natural attenuation predictions.

In this work, the long-term monitoring of BTEX/CAHs and microbial community in the groundwater of a pesticide plant was conducted. A machine learning approach for natural attenuation prediction was developed with RFC model followed by either RFR or ANN model, utilizing both microbial information and contaminant attenuation rates for model training and cross-validation. The performance of the models in the prediction of the feasibility and/or rates of natural attenuation of BTEX and CAHs was subsequently evaluated and compared. Results showed that the RFC model could achieve high accuracy (above 99%) for the feasibility prediction of natural attenuation for both BTEX and CAHs, and could successfully identify the key functional microbial genera. The RFR model was sufficient for BTEX natural attenuation rate prediction, and the MAPE was 8.1% ~ 19.6%. However, the predicted natural attenuation rates of CAHs by RFR were unreliable, with the MAPE as high as 22.0% ~ 153.9%. The ANN model showed better performance in the prediction of attenuation rates for both BTEX (with MAPEs from 2.1% to 4.6%) and CAHs (with MAPEs from 6.9% ~ 19.7%). Based on the assessments, a composite modeling method of RFC and ANN models was proposed, which could reduce the MAPE to as low as 2.1% ~ 4.6% for BTEX and 6.9% ~ 19.7% for CAHs. This study reveals that the combined machine learning approach under synergistic use of site microbial data has promising potential for predicting natural attenuation.

3.13.P-We258 In-Situ Remediation of Contaminated Sediments in Open Waters and Wetlands

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Over the past two decades there have been several developments in in-situ remediation of soils and sediments for petroleum hydrocarbons (including PAH compounds), PCBs, dioxins, and metals (especially lead and arsenic). In-situ treatment amendments have included activated carbon (granular and powdered), organoclay, highly concentrated microbial inoculants for bioremediation, and geochemical modifications for reducing metal bioavailability. This paper provides an overview of the developments using case studies for PCBs, dioxins, and lead. In-situ remediation for PFAS in soils and sediments is relatively new, and the presentation will provide a status. Because in-situ remediation involves a range of considerations including addressing potential for adverse impacts and addressing factors affecting efficacy, the utility of in-situ remediation methods for soils and sediments will be presented within the broader context of remedial decision-making.

3.13.P-We259 Unravelling Steroid Hormone Transformation Pathways in a Natural Wastewater Treatment System Through High-Resolution Suspect Screening

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Steroid hormones are environmentally relevant endocrine disruptors that undergo extensive biotransformation during wastewater treatment, yet most of their transformation products (TPs) remain unidentified. Natural treatment systems such as macrophyte pond–constructed wetland configurations are increasingly adopted as low-energy solutions, but their ability to degrade or generate TPs is still unclear. This study provides the first comprehensive characterisation of TPs derived from testosterone, levonorgestrel, and cortisone in a full-scale natural system treating urban wastewater.

Using a highly selective molecularly imprinted SPE protocol combined with ultra-high-performance liquid chromatography and high-resolution quadrupole time-of-flight mass spectrometry, we applied a suspect-screening workflow integrating accurate mass, MS^E fragmentation, elemental composition, and automated spectral matching. More than 30 TPs were detected across influent, intermediate, and effluent samples, revealing a complex network of reductive, oxidative, hydroxylation, and ring-cleavage pathways. Aeration strategies implemented in the pond–constructed wetland system markedly altered transformation dynamics. Testosterone was efficiently removed under aerobic conditions, with enhanced formation of oxidised and ring-opened intermediates. Levonorgestrel showed no detectable parent compound but produced persistent hydroxylated and reduced TPs, indicating stable precursors or strong sorption–desorption behaviour. Cortisone generated numerous polyoxygenated derivatives, many structurally novel and tentatively annotated, several of which persisted through the final effluent.

Overall, the system demonstrated a strong capacity to transform steroid hormones, but also produced a chemically diverse suite of TPs with varying persistence. These findings highlight that “removal” of parent compounds does not necessarily equate to detoxification and reinforce the need to evaluate TPs when assessing the performance and environmental safety of nature-based treatment technologies.

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3.13.P-We260 Enhancing Steroid Hormone Removal in a Natural Wastewater Treatment System Through Biosorbent Substrates and Aeration

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Steroid hormones are potent endocrine disruptors frequently detected in municipal wastewater effluents at concentrations capable of inducing adverse effects on aquatic biota, even at the ng·L⁻¹ level. Their recognised ecological relevance has led the European Commission to include key representatives in the Water Framework Directive and successive Watch Lists, highlighting the need for treatment technologies capable of achieving near-complete removal. Natural wastewater treatment systems (NWTs) offer a sustainable and low-energy alternative for small or isolated communities, but their performance regarding the full spectrum of steroid hormones remains insufficiently resolved.

This study investigates the presence, removal, and optimisation potential of steroid hormones in a full-scale macrophyte pond–constructed wetland system located on a university campus. Fifteen hormones from all major families were monitored over five months at three treatment stages. Under baseline operation, nine compounds—including testosterone, boldenone, estrone, 17β-estradiol, progesterone, megestrol acetate, cortisone, prednisone and prednisolone—were detected in influent waters at concentrations from 10 to 600 ng·L⁻¹. The facultative pond achieved removals >85% for most compounds, and only five hormones persisted in the final effluent at <30 ng·L⁻¹, demonstrating the intrinsic capacity of NWTs to attenuate high-impact contaminants.

Two enhancement strategies were evaluated: (i) replacing traditional substrates with locally sourced palm-tree residues (*Phoenix canariensis*), and (ii) continuous aeration of constructed wetland channels. The biosorbent substrate provided retention comparable to conventional media, offering a circular-economy alternative with minimal degradation after months of operation. Aeration yielded the most substantial improvement, driving the

complete elimination of nearly all detected hormones, strongly suggesting stimulation of aerobic biodegradation pathways.

These findings confirm that optimised NWTs can achieve removal efficiencies aligned with EU environmental policy objectives and can represent a robust option for regions with limited energy or infrastructural capacity. The demonstrated effectiveness of aeration and low-cost biosorbents provides a practical path for upgrading existing nature-based systems to meet future regulatory thresholds for endocrine-disrupting compounds.

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3.13.P-We261 A site-specific diagnostic framework for immobilization of metals and metalloids using dolomite-modified biochar in alkaline soils

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Soil amendments, such as biochar, are increasingly used to stabilize metals and metalloids to sustain healthy soil functions. However, their sorptive performance is highly site-specific, with strong controls by soil pH, carbonate-buffering capacity, organic matter, and Fe/Mn (oxyhydr)oxide content. Here, we evaluated a dolomite-modified biochar (DMB) (Carlini et al., 2021, Patent No. 102021000014195*), produced by co-pyrolysis of wood and dolomite, as a low-cost, mineral modified amendment to support in-situ immobilization of metals and metalloids in nine contaminated Austrian soils, differing in pH, total organic carbon (TOC), total inorganic carbon (TIC), Fe/Mn oxides, and contaminant load. Harmonized batch leaching and column percolation tests were used to quantify amendment effects on trace metals (Co, Ni, Zn, Cd, Tl, U, Cu, Pb) and oxyanions (As, Sb, Cr, Mo) relative to high temperature wood-based biochars and activated carbons. Across soils, trace metals were generally immobilized, but Cu and Pb showed pronounced, soil-dependent mobilization: Cu increased in organic-rich, weakly buffered soils via dissolved organic carbon-mediated complexation, whereas Pb was mobilized in carbonate-dominated soils with low TOC/TIC ratios and high total Pb, which is consistent with the formation of soluble Pb-carbonate/hydroxide complexes. For the oxyanions, DMB substantially reduced As and Sb leaching in carbonate-buffered, Fe/Mn-oxide-rich soils, but mobilized them in organic-rich, weakly buffered soils where elevated pH promoted DOC release and Fe oxide dissolution. Cr and Mo remained persistently mobile under alkaline conditions, highlighting a trade-off when multiple oxyanions co-occur.

From these patterns, we derived a diagnostic framework based on TOC, TIC (and their ratio), soil pH, Fe/Mn oxide content and total contaminant load to predict when carbonate-rich biochar will act as a beneficial, sustainable amendment and when it may pose mobilization risks. In suitably buffered alkaline soils, DMB can therefore stabilize residual contamination and support long-term ecosystem recovery, while emphasizing that heavily contaminated hotspots require prior removal or isolation.

*Carlini, C., Primante, A., Greggio N. (2021). Metodo per la fabbricazione di un materiale composito per il recupero di nutrienti e/o inquinanti da acque reflue, materiale composito ottenuto e relativo uso.

3.13.P-We262 EVALUATION OF SUNFLOWER CAPABILITY IN RECOVERING A MULTI-CONTAMINATED SOIL MIXED WITH POLYCHLOROBYPHENIL POLLUTED MARINE SEDIMENT

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Nature-Based Solutions have emerged as effective, sustainable and cost-effective strategies for recovering contaminated environments from organic and inorganic contaminants. Among these strategies, Plant-Assisted Bioremediation (PABR) represents a methodology which decontaminates soil, sediments and water via the synergistic interactions established between plants and rhizosphere microbial communities. Sunflower (*Helianthus annuus* L.) has demonstrated a high capacity to remove pollutants, especially heavy metals. In this

work, the capability of Sunflower (*Argentario* var) to decontaminate a multi-contaminated soil was assessed in greenhouse microcosms. For this purpose, aliquots of soil collected from a polychlorinated biphenyl (PCBs) and heavy metal (HMs) contaminated site were mixed with those of a PCB polluted marine sediment (from the Mar Piccolo). The area studied was located in Taranto (Apulia Region, Italy). The contaminated substrate was used for setting up microcosm experiments with sunflower in absence/presence of a municipal waste compost. Moreover, control microcosms (without plants) were also performed. Soil chemical-physical parameters (e.g. pH, EC, etc), contaminant analytical determinations and microbiological analyses (microbial abundance and dehydrogenase activity) were carried out at 45 and 90 days. Moreover, qPCR analysis was performed for assessing the microbial community composition and ecotoxicological tests were carried out to assess the efficacy of the PABR technology.

The main results obtained in sunflower planted microcosms evidenced that soil PCB concentration reduced at end of the experiment (90 days) strongly in presence of sunflower and compost, while in the unplanted microcosms PCB removal was observed only if the compost was added. Microbial abundance and dehydrogenase activity highly increased in all the sunflower planted microcosms and the highest values were in the planted microcosms treated with compost. Further investigations are on-going to characterize sunflower's root exudates and rhizosphere microbial community structure and composition.

3.13.P-We263 Nature-based Remediation of Metal Pollutants in Nature Areas (NARMENA): An Ecological Modelling Approach

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The EU-LIFE project NARMENA (Nature-based Remediation of Metal pollutants in Nature Areas to increase water storage capacity) aims to evaluate the efficacy of Nature-Based Remediation (NBR) techniques in mitigating metal pollution in soil and sediment within select nature reserves in Flanders. Focus is on three target sites that have been selected for their historical contamination with metals. The Grote Calie is contaminated with chromium, the Winterbeek is strongly contaminated with metals and chlorine, and the final site, the Grote Laak contains high levels of cadmium and arsenic.

NBR techniques were implemented at these target sites. At the Grote Calie, a combination of Bacteria-Assisted Phytostabilisation (BAP) and constructed wetlands (CW) was employed to reduce metal contamination. Specifically, applying BAP will reduce metal bioavailability by stabilising and/or fixating the metals in the soil, rhizosphere, or through absorption by plants and accumulation in (underground) plant biomass. CW were employed by flooding the land or keeping the contaminated areas below the water level, creating artificial wetlands. Submersion leads to reduction of metal ions in the soil which reduces metal bioavailability and immobilizes the contaminants.

To support the ecological impact assessment of remediation measures, an ecological modelling approach was developed. This tool allows to predict the impact of remediation techniques on the ecotoxicity of specific contaminants. The model was developed based on laboratory toxicity testing for three species (i.e., *Hyaella azteca*, a freshwater amphipod, *Lemna minor*, a macrophyte, and *Daphnia magna*, the water flea). The ecological modelling approach allows to simulate the influence of physicochemical parameters affecting bioavailability on populations of these three indicator species. The impact of different remediation scenarios at the target sites could be assessed, differentiating between no action, conventional excavation approaches, and NBR techniques (BAP and CW). The NARMENA tool provides insight into the potential of NBR techniques to effectively reduce metal pollution in nature reserves, while also promoting sustainability and improving local water retention. This tool highlights the added value of ecological modelling in supporting nature conservation and remediation initiatives.

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3.13.P-We264 Reducing Antibiotic Resistance Genes via vermiremediation in soils treated with Oxytetracycline, Sulfamethoxazole, and Enrofloxacin

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Antibiotic resistance genes (ARGs) are recognized as emerging microbial pollutants that threaten human and animal health by contributing to the spread of antibiotic-resistant bacteria and diminishing the efficacy of current antimicrobial treatments. This study assessed the capacity of *Eisenia fetida* to reduce ARGs associated with the antibiotics oxytetracycline (OTC), sulfamethoxazole (SMX), and enrofloxacin (ENR) in agricultural soil through a vermiremediation approach. Initially, environmentally relevant concentrations of these antibiotics (4.21 mg/kg OTC; 2.21 mg/kg SMX; 1.61 mg/kg ENR) and levels comparable to those found in animal manure (42.1 mg/kg OTC; 22.1 mg/kg SMX; 16.1 mg/kg ENR) were applied to soil under controlled laboratory conditions for 136 days to induce ARG development. Subsequently, *E. fetida* earthworms were introduced into the contaminated soils for 28 days to evaluate their remediation potential. Amplicon sequencing of 16S rRNA and high-throughput quantitative PCR (HT-qPCR) revealed that *E. fetida* modified both the soil bacterial community structure and the resistome. Dominant phyla across treatments included Actinobacteriota, Proteobacteria, Firmicutes, Planctomycetota, Verrucomicrobiota, and Gemmatimonadota. Among genera with relative abundance >0.5%, *Bacillus*, *Candidatus Udaebacter*, *Gemmatimonas*, JG30-KF-AS9, *Streptomyces*, and *Paenibacillus* were most prevalent. The highest antibiotic mixture stimulated an increase in genes conferring resistance to sulfonamides (*sul1*) and multidrug efflux systems (*acrA*, *tolC*, *mdtH*, and *mdtE/yhiU*). Vermiremediation mitigated all these genes except *mdtH*. Genera JG30-KF-CM45, KD4-96, and TK-10 showed positive correlations with most ARGs, suggesting they may act as reservoirs. In conclusion, these findings indicate that vermiremediation using *E. fetida* is a promising strategy for reducing ARGs from multiple antibiotic classes in agroecosystems. Partially funded by Joint Research Laboratory on Environmental Antibiotic Resistance (Euskampus), “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES” (001) and “Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq” (307390/2017-9) Brazil. Funded by the Basque Government: CBET+: IT1743-22 Consolidated Research Group.

3.13.P-We265 Assessing the toxicity of phytoremediated mining soils on *Eisenia fetida* and *Lactuca sativa*

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Soil contamination by metals derived from mining activities represents a persistent environmental challenge due to their persistence, stability and toxicity. These pollutants accumulate in soils, creating long-term ecological risks. Phytoremediation has emerged as a cost-effective and environmentally friendly approach, and the use of sunflowers combined with pollutant-resistant bacteria is considered promising for enhancing metal uptake and plant resilience. However, its efficiency remains uncertain, as benefits vary across biological levels. To evaluate this strategy, mining soils from Lanestosa (Spain) and Sentein (France) were treated with sunflowers stabilized with bacterial inoculants. Six treatments per site were applied under blind conditions with 4 replicates each. Toxicity was assessed at organism level using *Eisenia fetida* and *Lactuca sativa* through soil and leachate bioassays, and at cellular level via coelomic fluid analyses (cell viability and fluorescence biomarkers: riboflavin and calcein). These indicators provide complementary insights into oxidative stress and membrane integrity. Results showed no significant differences in earthworm survival or weight change, and lettuce germination exceeded 70% across treatments, suggesting low acute toxicity. Root elongation varied within sites but lacked consistent effects related with treatment. Cellular assays revealed more nuanced responses: riboflavin concentrations were higher in bacterial inoculation treatments, suggesting reduced oxidative stress, whereas calcein fluorescence indicated greater membrane stability in untreated soils after 14 days. These contrasting trends imply that bacterial inoculation may mitigate certain stress pathways without uniformly improving soil quality. Overall, organism-level endpoints suggest limited remediation benefits, while cellular biomarkers highlight complex physiological interactions. Although phytoremediation supported by bacteria shows potential for reducing sublethal toxicity, its effectiveness as a comprehensive remediation strategy remains inconclusive. Future research should extend exposure periods, integrate soil physicochemical analyses, and refine bacterial consortia to enhance ecological relevance and optimize remediation outcomes. Funded by: PhytoSUDOE-SOE1/P5/E0189. *A. Brückner, A. Fow, and M. Mehlhop are supported by the Erasmus+ Student Scholarship ECT+ EMJMD "European MSc in Environmental Contamination and Toxicology" 2022-2024.

3.13.P-We266 Anaerobic biodegradation by microbial fuel cell of the insecticide imidacloprid for the production of sustainable energy.

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The Imidacloprid is an insecticide widely used worldwide in agricultural activities to combat pests. Its extensive use has raised concerns regarding the environment due to contamination of soil and water bodies, through soil washout by runoff and atmospheric deposition by spraying. So, in recent years, the need for studies that promote the generation of clean energy through sustainable means has grown. This study aims to develop a microbial fuel cell using the bacterium *Shewanella oneidensis* for the treatment of wastewater contaminated with the insecticide by oxidative cleavage and that is capable of generating sustainable energy as a product. A double-compartment microbial fuel cell was constructed with an anode containing the bacterial biofilm and a cathode, both consisting of platinum separated by a Nafion proton exchange membrane and a stainless steel current collector. The treatment of a solution containing 100 mg.L⁻¹ of imidacloprid was performed in triplicate for three days with an external resistance of 220 Ω. Then, 5 mL of the treated samples underwent an organic phase extraction for subsequent analysis of the by-products generated and compared with the control samples by gas chromatography coupled to mass spectrometry (GC-MS). For this, this study too develop and validate analytical methodologies for the efficient detection and quantification of imidacloprid and its metabolites in anaerobic biodegradation by microbial fuel cells using gas chromatography coupled with mass spectrometry (GC-MS). Was carried out 32 different approaches were analyzed at concentrations of 10, 5, and 0.5 mg/L using a commercial standard of imidacloprid. However, through laboratory validations, only 8 methods were refined and tested at lower concentrations (0.3-0.1) mg/L. Among them, only one method was selected based on the peak integration area, column preservation, absence of baseline noise in the chromatogram and energy efficiency. The collected samples from the fuel cells were subjected to an organic phase extraction process using dichloromethane (CH₂Cl₂) with the purpose of extracting imidacloprid and its possible by-products from the reaction medium, such as Imidacloprid-urea. Subsequently, the samples were rotary evaporated and dissolved in acetone prior to GC-MS analysis. The chromatographic results confirmed the efficiency of anaerobic biodegradation within microbial fuel cells, demonstrating its potential for IMI degradation and environmental remediation.

Disclaimer/Disclosure:

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3.13.P-We267 Integrating Molecular and Geochemical Evidence of Biodegradation during Bioremediation of a Linear Alkylbenzene-Impacted Aquifer with a tailored native microbial consortium
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In 2016, the aging of a high-voltage cable resulted in the release of more than 30,000 L of insulating dielectric oil rich in linear alkylbenzenes (LABs) into a fractured coastal calcarenite aquifer in Spain. After light non-aqueous phase liquid (LNAPL) physical removal, a bioaugmentation approach using a native tailored microbial consortium and aerobic biostimulation were implemented to treat the contaminant plume.

A specialized LAB-degrading microbial consortium was enriched from the indigenous microbiota (GWP-F1) demonstrating complete degradation of the dielectric oil components. After detailed community analysis, consortium GWP-F1 was scaled up for field application.

Following successful pilot testing, a full-scale bioaugmentation strategy was implemented consisting in a series of groundwater recirculation cells equipped with infiltration–extraction wells connected to an aeration system enabling nutrient addition and oxygenation of the recirculated water to support biostimulation. Treatment cycles consisted of oxygenation followed by nutrient and inoculum injection.

Microbial dynamics and biodegradation processes were monitored through MPN counts, qPCR targeting key consortium phylotypes (*Sphingobium*, *Hydrogenophaga*, and *Gordonia*), and functional genes involved in LAB degradation (P450 cytochrome monooxygenases, AlkB alkane hydroxylases, and biphenyl/toluene dioxygenases). Changes in microbial community structure were evaluated using 16S rRNA amplicon sequencing. Hydrocarbon degradation was further assessed through GC-MS analysis of LAB isomer-specific diagnostic ratios.

The bioaugmentation strategy resulted in substantial reductions in groundwater LAB concentrations and induced a pronounced restructuring of the microbial community. Targeted consortium-associated 16S rRNA biomarkers and functional genes exhibited a robust enrichment of 1–2 orders of magnitude across the treated areas,

maintaining stable abundances over time. GC-MS analysis confirmed a significant reduction in the most biodegradable LAB isomers, providing strong and consistent evidence of active in situ biodegradation.

3.14.P Improving Understanding and Modelling of the Partitioning, Mobility, and Bioavailability of Highly Polar and Ionisable Substances in the Environment

3.14.P-Th061 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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Several studies have applied screening level assessment approaches to identify substances that could potentially be mobile in the environment. Due to the lack of suitable alternative metrics, these prioritisation exercises have generally been based on the organic carbon normalised partition coefficient (KOC), considering it the principal indicator of substance mobility in the environment. However, concerns have been raised about the appropriateness of the KOC concept to indicate the potential for substances to be mobile in the environment. The partitioning and binding of substances to components of soils and sediments is an important aspect of their environmental fate and can determine both their mobility and bioavailability. However, tests using defined natural soils do give variable results due to the extremely diverse nature of soil characteristics in terms of their contents of organic carbon, clay, and iron and aluminium (oxy)hydroxides, as well as their cation exchange capacity and pH. There are three principal mechanisms that contribute to the overall retention of substances in soils and other sub-surface media. These are phase partitioning, cation binding, and anion binding. There are several components of soils and other subsurface media that can be involved in one or more of these mechanisms; for example soil organic matter is important in phase partitioning for hydrophobic and nonpolar substances, but can also be important for the binding of cations. The extremely high variability of the overall distribution coefficient KD between different soils limits its usefulness as an inherent measure of the environmental fate of a substance. It may be possible to define partition coefficients for interaction mechanisms, such as cation or anion exchange (e.g., producing KCation and KAnion, respectively) that could then be applied collectively to consider all of the individual partitioning mechanisms, including the KOC value, to derive an overall partition coefficient. The need to include characteristics of the medium will improve the relevance of partitioning information for hazard and scenario-specific exposure assessment purposes, although it will also require that reference conditions are defined to allow comparisons to be made between different substances. Improved approaches are likely to need to take account of the local environmental conditions, and this will improve the relevance of the information especially for site-specific risk assessments.

3.14.P-Th062 Experiments on the Retention Behaviour of Ionic Organic Substances on Mixed-Mode HPLC Columns for the Future Expansion of OECD 121

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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 investigated whether state-of-the-art mixed mode stationary phases could 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. Following a method development phase, tests were performed with 56 substances using strong cation exchange (SCX) and weak cation exchange (WCX) columns, both combined with reversed phase material. Retention of the cationic test substances was observed; the results are promising and indicate that we could develop an extension to the OECD TG 121. This multi-year project is intended to provide a step towards a possible solution to improve the mobility assessment of polar and ionic substances in low organic carbon soils and subsurface media.

3.14.P-Th063 Evaluation of Prediction Methods for the Mobility Criterion KOC

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With the new PMT (persistent mobile toxic) classification of chemicals by the EU since 2024, the partition coefficient between organic carbon and water K_{oc} received increased attention. Several prediction tools for this property have been developed. The Technical Guidance Document for REACH (TGD) lists log K_{ow} – log K_{oc} regressions for various substance groups. For acids and bases, Franco and Trapp developed regressions using pH of soil. A regression by Ecetoc uses log D as predictor for the adsorption of bases, while that of Droge and Goss for cations relies on molar volume and the number of hydrogen atoms at the charged nitrogen. Li et al. developed six new regressions for the distribution coefficient K_d of acids, weak and strong bases and neutral chemicals. The K_{oc} was also estimated by the machine learning tool implemented in Opera, listed in CompTox. Using experimental data sets from literature, we tested accuracy (sum of residues SU) and precision (log mean absolute error log MAE) of these prediction methods for neutrals chemicals, acids, bases and zwitterions. The experimental data set of Li et al. (2021) contains 689 measured K_d-values for 54 chemicals (pharmaceuticals, half of them bases) in eight soils and five sediments. Soils and sediments were characterized by an array of data, such as pH, TOC, CEC, %clay, %silt, and content of K, Mg, Na.

The K_{oc} of neutral pharmaceuticals was well predicted except for a few polar chemicals (about factor 10 average error). Adsorption of acids was even better predicted, best by the regression equation of Li et al. with less than factor 3 absolute error. The K_{oc} of bases, mostly present at cations, was widely underpredicted and with less precision (0.82 ? log MAE ? 1.40). Zwitterions were among the chemicals with the highest and the lowest K_{oc} and those with the widest range. No method could predict adsorption of zwitterions satisfyingly (avg. error > factor 25). CompTox gave good K_{oc} values for the bulk of chemicals but had a few high outliers, which increased average error and lowered reliability.

Among properties of the soil, pH had the most significant impact on K_d and K_{oc}, followed by TOC and CEC. The choice of method depends on the availability of such input data: methods requiring more data generally performed better, but those data are not always at hand, while generic estimation methods, such as CompTox/Opera or the TGD regressions, can be used without knowledge of the soil properties.

Disclaimer/Disclosure:

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3.14.P-Th064 An Approach for Accounting for Structural Similarity in Reference Substance Selection for Highly Mobile Test Substances in HPLC based Fate Studies

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In this presentation, we outline an approach for applying structural similarity profiling to improve the selection of reference substances for screening tests of the environmental partitioning and mobility of substances using HPLC based methods calibrated with reference substances. The structural similarity profiling approach uses publicly available tools, such as the OECD QSAR Toolbox, so that structurally similar reference substances can be identified for test substances to use for calibration of the methods, potentially enhancing the reliability and relevance of KOC and mobility testing for chemicals. Hypothetical examples are used due to the lack of reliable partitioning data for these substances.

Seventeen ionizable cationic substances with the potential for high mobility in the environment were selected for assessment. Because no distinction between reference and test substances was possible, a subset of the test

substances were tested together, and the similarity of each substance in the suite was assessed against two of the individual substances, propranolol and tryptamine. To evaluate the applicability of using the OECD QSAR Toolbox for this purpose, an initial structural similarity assessment was conducted.

The reference substances were tested against a set of potential test substances using the Dice coefficient similarity measure and the PubChem molecular features system.

Substances were considered to be potential reference substances where they had a structural similarity of at least 60% when compared to the hypothetical test substances. For propranolol six potential reference substances were identified (2,2'(benzylimino)diethanol, atenolol, benzyltrimethylammonium chloride, lidocaine, methylephedrine, and serotonin). For tryptamine seven potential reference substances were identified (2,2'(benzylimino)diethanol, benzyltrimethylammonium chloride, ciprofloxacin, cotinine, florasulam, lidocaine, and serotonin). This demonstrates that it is possible to evaluate the structural similarity of test and reference substances using free publicly available software. However, given the limited number of substances for which reliable environmental partitioning information is available the application of this approach to identify suitable reference substances could significantly reduce the number of reference substances available.

3.14.P-Th065 Development of column testing approach for the mobility assessment of chemicals during river-bank filtration.

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River-bank filtration (RBF) is a widely used technique which utilizes the natural filtration capabilities of sediment and gravel to remove impurities and contaminants in the extracted water. However, there are potential risks, as certain non-degradable and highly mobile chemicals can pass through the filtration system and contaminate the drinking water sources. Existing regulatory tests do not include methods to evaluate the mobility of polar chemicals in usually very low/no organic carbon RBF materials. This study aimed to create a column testing approach with aquifer materials accompanied by a transport modelling approach to assess the mobility of polar chemicals during RBF.

In this study, two sandy aquifer materials from Haltern am See and Berlin (Germany) with variable cation exchange capacity, content of organic carbon, texture and porosity were selected. Three test substance 1) nitrophenol (classified as potential PMT & vPvM)1, 2) melamine (classified as PMT & vPvM)1 and 3) carbamazepine (detected in groundwater and drinking water)1 were used in this study.

A column test setup with water saturation in up-flow condition similar to DIN 19528 was used as a basis for the development of this testing approach. For this study, columns with diameter 14 cm were filled with aquifer material to a height of 40 cm and were initially saturated with tap water. A flow rate of 1.1 mL/min was used and the test was conducted at 20°C in the dark. As a first step, the test substance and the tracer (KBr) were loaded as eluent (dissolved in tap water) into the saturated column at substance concentrations of 0.1 mg/L and tracer/KBr concentration of 0.2 mg/mL. This loading step was carried out for 3 d and was followed by a washing step where the eluent was exchanged with tap water that was flushed through the column for 2 d at a similar flow rate. For both loading and washing steps, the concentrations of test substances along with other phys-chem parameters of the eluate such as pH and electrical conductivity were measured. The results obtained from these column studies will be presented.

References

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3.14.P-Th066 A workshop to engage with stakeholders, identify data gaps and establish research priorities for the development of a screening test to assess the mobility of polar and ionisable substances

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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 can determine both their mobility and bioavailability in the environment. Screening level assessment approaches to identify and prioritise potentially mobile substances are generally based on the organic carbon normalised partition coefficient (KOC) as the principal indicator of substance mobility; this is because organic matter plays a dominant role in the adsorption of many substances to surface soils. The OECD Test Guideline (TG) 121 screening method for the measurement of KOC benchmarks the relative retention time of a test substance with a series of reference substances on an HPLC system using a cyanopropyl stationary phase; this allows for the interaction of polar and non-polar groups of a test substance but 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 contribute to the overall retention of substances in soils and sub-surface media. Following an evidence review and experimental phase investigating the potential to extend the general concept of the OECD TG 121, a workshop was undertaken to engage with stakeholders, secure support, identify data gaps and uncertainties, and understand the best way forward with this work. The workshop included a series of presentations and breakout groups, which encouraged discussion and comment, on the historical background of the OECD TG 121, the evidence review, the experimental work and potential next steps. In general, an extension to the OECD TG 121 that enables the consideration of interactions between substances and components of soils and sediments, beyond those associated with organic carbon, was supported. Key outcomes included increased awareness and interest in moving the work forward; external influence on the project direction; clarity on the data gaps and experimental work that is needed; and potential collaborations to progress this work. The workshop has provided the basis from which a road map for future development can be established. Presenting this workshop will provide a valuable learning exercise in how to work with key stakeholders to improve regulatory acceptance.

3.14.P-Th067 Taking Account of Bioavailability in Assessing the Potential Risks Posed by Diclofenac in European Surface Waters

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The nonsteroidal anti-inflammatory drug diclofenac has widespread use and can enter the environment via wastewaters. Diclofenac is a monocarboxylic acid with a pKa value of approximately 4, meaning that at pH 4 diclofenac will be present in approximately equal amounts in both ionised and unionised forms. At pH 6 approximately 99% of diclofenac will be present in the ionised form, and at pH 7 approximately 99.9% of diclofenac will be ionised. The physicochemical properties of diclofenac indicate that the substance is water soluble, ionised in aqueous environmental media, and unlikely to undergo significant environmental partitioning due to its presence in an anionic form in the environment. Diclofenac environmental behaviour is therefore typical of many other human pharmaceuticals. It readily ionises, is water soluble, and shows a limited tendency to partition to environmental matrices, which is unsurprising as these are often clinical design requirements for medicines. It has recently been proposed that Environmental Quality Standards (EQS) for diclofenac should take account of its ionisation, and consider differences in the uptake and toxicity of the ionised and unionised forms. Normalisation of toxicity data for diclofenac based on pH has been reported to reduce heterogeneity in acute data and improve the Species Sensitivity Distribution (SSD) of chronic data. Bioavailability based approaches for EQS derivation are usually based on the normalisation of toxicity data to a consistent set of water chemistry conditions, and the calculation of a site-specific SSD. However, in cases where all species in the ecotoxicity database use the same bioavailability normalisation approach it may be possible to apply a bioavailability correction to an HC5 value derived from a bioavailability normalised SSD. Water chemistry conditions vary widely across Europe, and EQS values that take account of this variation have been set within Europe for over a decade and are now widely implemented. This presentation considers the variation in local pH conditions of surface waters across Europe to identify differences in the relative sensitivity of areas to diclofenac toxicity.

3.14.P-Th068 Prediction of the mobility of gabapentin, tramadol, diclofenac, acesulfame, and methamphetamine in soils based on their properties

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Agricultural soil can be contaminated by various micropollutants. One source of these micropollutants is contaminated river water or even treated wastewater used for irrigation, or sludge from wastewater treatment plants used to enrich the soil with organic matter and nutrients. Compounds occurrence in soil 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. The five compounds, i.e., gabapentin, tramadol, diclofenac, acesulfame, and methamphetamine, that are frequently found in the water environment were selected. To determine their behavior in different soil environments, standard sorption experiments were performed for each of them in eight 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, e.g., organic carbon content, clay content, soil pH, cation exchange capacity, and wettability index. These equations and the maps of soil properties were used to predict the KF value distributions within the agricultural soils of the Czech Republic and subsequently to delineate classes of compounds' mobility in the soil environment. The standard dissipation tests in three soils were also conducted to determine their persistence in soils. 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-Th069 Testing the Applicability of Diffusive Gradients in Thin-Films (DGT) to Assess Pesticide Bioavailability in Soil

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The growing global demand for food results in an increased use of pesticides in agricultural production to ensure corresponding crop yields. Once applied, pesticides enter the environmental matrix of soil, where they undergo a range of processes e.g., sorption, microbial degradation, and ageing. In conventional risk assessment, the total extractable concentration of pesticides in soil is typically used as the standard for exposure. However, this method anticipates the most adverse circumstances and does not account for environmental processes that modify pesticide fate and thereby influence their (bio)availability. Consequently, determining pesticide bioavailability is essential for risk assessment, remediation strategies, and related decision-making. In this project, we focus on determining pesticide bioavailability using passive sampling. Although the diffusive gradients in thin-films (DGT) technique has demonstrated strong validity and performance for a wide range of metals, metalloids, and other inorganic substances, its application to organic compounds such as pesticides remains relatively novel. Therefore, while the use of DGT for determining pesticide bioavailability in soil might be an innovative extension of this method, it still requires thorough investigation. We will evaluate DGT performance under controlled conditions with water-saturated soils, compare it with other passive sampling techniques (e.g. silicone-based materials) and test whether it provides valid results as a proxy for the actual pesticide exposure. If so, current risk assessment frameworks may get more environmentally meaningfully refined.

3.14.P-Th070 Leaching of heavy metals, nicotine, and other organic compounds from spent disposable e-cigarettes and their environmental implications

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The use of disposable e-cigarettes is widespread and increasing globally. Disposable vape products are designed to be single-use devices that are discarded after the e-liquid is exhausted. Due to a lack of standardized recycling procedures, most spent disposable e-cigarettes end up in municipal waste. The hazardous chemicals leaching from these spent disposables into landfills and the nearby surrounding environment pose significant environmental concerns. This study aims to characterize the leaching of heavy metals, nicotine, other polar organic contaminants, and potential nicotine transformation products from the spent disposable devices (NJOY Menthol) in different aqueous environments at different pH, including (1) deionized water, (2) synthetic leachate, and (3) simulated rainwater. Samples were collected at six time points, every 48-72 hours, spanning from 0 to 11 days. Environmental matrices influenced the leaching rates, causing an increase in nicotine,

menthol, and heavy metals over time in all conditions. Nicotine reached a plateau in both synthetic leachate and simulated rainwater conditions, whereas the observed increase of nicotine and anabasine could be explained by possible transformations into other forms of nicotine. These findings indicate a potential rise in pollution and ecotoxicity associated with improper disposal of the spent disposable e-cigarette devices and highlight urgent needs for regulatory measures governing the use of these products.

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3.15.P Innovative Approaches to Groundwater Quality Monitoring: From Detection to Policy

3.15.P-Th071 Relationship Between the Occurrence of Organic Micropollutants and Groundwater Conditions in Drinking Water Aquifers: The Netherlands as a Case Study

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The occurrence of organic micropollutants (OMPs) in drinking water aquifers has been widely documented. Physiochemical, hydrogeological and biological processes influence the fate of OMPs in the environment, mainly through biodegradation and sorption. Biodegradation and sorption potential is OMP specific, through differences in physio-chemical properties of OMPs interacting with groundwater chemistry and the soil matrix. OMP occurrence in drinking water aquifers is a complex interplay of different mechanisms, and it is challenging to predict the prevalence for a given aquifer. This research aimed to study relationships among the occurrence of OMPs in drinking water aquifers and groundwater conditions as well as the role of OMP-specific properties, with the Netherlands as a case study. The data set covered 286 individual extraction wells distributed over 23 extraction fields in the eastern and northern part of the Netherlands. Macrochemistry data (pH, electron acceptor and dissolved organic carbon (DOC) concentrations) and OMP concentrations were measured by a Dutch drinking water company over a 9-year period (2016 to 2025). This data shows that pesticide transformation products are detected most at extraction wells. 2,6-dichlorobenzamide (BAM), transformation product (TP) of the pesticide dichlobenil, is the most frequently detected compound at the extraction wells (46%). The TPs of other pesticides also have a higher presence than their parent compounds, showing the persistence of these TPs. The data shows that OMP charge state is predictive for OMP occurrence, with a lower occurrence of positively charged OMPs (N = 15 wells) than neutral (N = 245) / negatively charged (N = 223) OMPs. Electrostatic interactions, such as attraction between positively charged OMPs and negatively charged soil particles is a process likely to contribute to limited transport of positively charged OMPs. Lastly, the data shows a positive trend between the concentration of DOC (mg/L) and summed concentration of PFAS (ng/L) in individual extraction wells, if PFAS is detected. These new insights on the factors influencing OMP occurrence at extraction wells contribute to improved prediction and management of OMPs in drinking water sources.

3.15.P-Th072 PFAS Levels in Soil and Groundwater Profiles: Implications for Drinking Water Production from Groundwater

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Per- and polyfluoroalkyl substances (PFAS) are highly fluorinated chemicals that have been produced and used on a large scale in various industries and products since the 1950s. During the production and application of PFAS, these substances have also been diffusely distributed via the air and associated atmospheric deposition. As a result, they are present almost everywhere in the soil at low concentrations. A significant part of the total PFAS load is still present in the unsaturated zone of the subsurface. From the unsaturated zone, PFAS migrate to the groundwater, which is extracted for drinking water production. However, there is still insufficient generic knowledge about the presence and distribution of PFAS in the soil, and how and when PFAS will spread to drinking water sources that utilize groundwater. This is especially true for the relatively low concentration ranges which are found in diffusely PFAS-contaminated areas. In this study, the presence and fate of PFAS in the subsurface is further researched.

The monitoring part of the study maps the concentration depth profile of 34 PFAS in the unsaturated zone and in groundwater. For this, samples of soil in the unsaturated zone were taken at 101 locations at multiple depths. Additionally, samples of groundwater at multiple depths were taken at 24 locations. There were approximately

seven filters per well, with depths ranging between 3 and 10 m below surface level.

The results of the monitoring study confirm that legacy PFAS in the subsurface are still largely contained within the unsaturated zone. This raises further questions about the dominant transport processes of PFAS in the subsurface. If the peak of PFAS production and atmospheric deposition was in the past, the largest peak would not have been in the topsoil but a bit further down. Therefore, the results will be used to improve modelling by providing an estimation of the environmental behavior of PFAS in the soil and of the diffuse PFAS load that is still on its way from the soil to ground- and drinking water.

3.15.P-Th073 Uncovering PFAS in Groundwater: Occurrence, Hotspots and Health Risks Implications

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Groundwater is a key resource for urban and agricultural water supply, yet its quality is increasingly threatened by industrial, urban and agricultural activities, and by over-extraction that may enhance pollutant mobilization. Emerging contaminants (ECs), particularly per- and polyfluoroalkyl substances (PFAS) raise particular concern due to their persistence, mobility and potential health impacts. Upcoming revisions of the EU Water Framework Directive and Groundwater Directive, including the establishment of a Groundwater Watch List (GWWL), reflect the need for harmonized monitoring and regulation across Europe.

To support these initiatives, the Catalan Water Agency (ACA) implemented a regional monitoring network to assess the occurrence, hotspots and potential risks of relevant ECs, including PFAS and pharmaceuticals (PhACs), in groundwater. Between 2022 and 2024, 94 groundwater sites impacted by urban and industrial activities across Catalonia were analyzed. Twelve PFAS, including perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSA), and eleven PhACs, were quantified using UHPLC-HRMS and UHPLC-MS/MS, respectively. Environmental risks were evaluated using hazard quotients ($HQ = MEC/PNEC$) using data from the NORMAN database, while human health risk followed the EFSA guidance based on the summed intake of four PFAS (PFOA, PFNA, PFHxS and PFOS).

PFAS were widespread, with detection frequencies above 89% for PFPeA, PFOA and PFOS. Concentrations ranged from sub ng/L to several hundred ng/L, reaching 773 ng/L for PFOS. Short-chain PFCAs and PFSA dominated, particularly near industrial areas. Many sites exceeded the 100 ng/L threshold for the sum of 20 PFAS set in Spanish drinking water regulations. PhACs such as carbamazepine, sulfamethoxazole, amidotrizoic acid and primidone were also detected, sometimes above 100 ng/L, and even exceeding 1000 ng/L for amidotrizoic acid. Environmental risk assessment revealed moderate to high risks ($HQ > 0.1-1$) at many sites, primarily driven by PFOA and PFOS. Human health assessment showed that summed HQs for the four PFAS often exceeded 1, with some surpassing 10, indicating potential health concerns if groundwater were used for consumption of drinking water without treatment. Overall, PFAS are the most critical contaminants due to their ubiquity, persistence and strict regulatory limits, highlighting the need for harmonized monitoring, pollution control and targeted groundwater remediation.

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3.15.P-Th074 A Comprehensive Assessment Framework for Chemical Mixtures in Urban Groundwater

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Urban groundwater (GW) is increasingly considered a strategic alternative water resource for cities facing population expansion, recurrent droughts, and intensification of climate change effects. However, its suitability for drinking purposes remains uncertain due to the presence of complex mixtures of organic contaminants. This study presents a comprehensive assessment framework that integrates target analysis, suspect and non-target screening (NT/SS), human-health risk evaluation, and numerical mixing modelling to characterize and prioritize contaminants in an urban aquifer. In this context, six monthly sampling campaigns were conducted collecting 48

GW samples from eight wells/piezometers in the Poble Sec pilot zone of Barcelona (Spain), an area where oxic aquifers are strongly influenced by urban activities. Samples were concentrated and analysed by LC-HRMS, enabling the quantification of 123 target compounds spanning pharmaceuticals, industrial chemicals, pesticides, transformation products, food additives, and drugs of abuse. Complementary, NT/SS is ongoing to explore additional substances/candidates not included in the target list. Overall, 108 compounds were detected at least once, and 45 were ubiquitous across all campaigns, suggesting a chemically diverse mixture influenced by multiple urban sources. Total concentrations were dominated by industrial chemicals, followed by pharmaceuticals and food additives, with compounds such as tris(chloropropyl) phosphate and melamine capable of migrating and persisting through the subsurface. Spatial and temporal patterns indicated contamination hotspots as well as attenuation processes along the flow path. Human-health evaluation using consumption rates posing health risk revealed the presence of potential risk compounds (e.g., Docusate or Pentobarbital) that fell into high-priority categories, whereas most contaminants exhibited values related to low concern under typical GW consumption scenarios. Finally, a mixing-modelling employing MIX code is being applied to quantify recharge-source contributions and prioritize contaminants as potential tracers in the urban aquifer. This multi-tiered approach (combining chemical screening, risk prioritization, and modelling) provides a robust basis for assessing the implications of chemical mixtures in GW and supports its future evaluation as a complementary drinking-water resource.

Keywords: Organic Contaminants, Urban aquifers, Chemical Screening, Risk Assessment

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3.15.P-Th075 Characterising Emerging Contaminants in a Groundwater Catchment Using Advanced Screening Approaches

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Groundwater is essential for sustaining urban, agricultural and ecological needs, especially in Mediterranean regions facing prolonged droughts. Reduced recharged and intensified pumping may alter stream-aquifer interactions and open new pathways for emerging contaminant (EC) infiltration, such as pharmaceuticals (PhACs), endocrine disrupting chemicals (EDCs), PFAS, pesticides and other anthropogenic chemicals. These ECs reach aquifers at low ng/L levels, challenging groundwater quality assessment. Recent advances in European groundwater policy, that introduce new quality standards for some ECs, underscore the need for monitoring approaches that capture a broader spectrum of ECs. While targeted analysis may underestimate contaminant occurrence, HRMS-based suspect screening can detect a wider range of ECs, and their combined application offers a more comprehensive assessment framework. In this context, the present study integrates advanced target and suspect screening methods to characterise ECs in the drought-affected Onyar River aquifer, and to demonstrate the added value of using these combined strategies for groundwater monitoring.

Groundwater and river samples were collected during two campaigns (2021–2023) under contrasting hydrological conditions. Target LC–MS/MS analysis focused on 45 PhACs, 9 metabolites and 32 EDCs, while HRMS-based suspect screening (Orbitrap Exploris 120) was applied to broaden chemical coverage. Target analysis detected only a small subset of compounds, mainly PhACs and selected EDCs, typically below 20 ng/L, with higher concentrations in sites near the river and attenuation was observed at depth. In contrast, suspect screening revealed a much wider chemical profile, identifying 67 compounds including additional PhACs, metabolites, pesticides, and multiple transformation products, indicating active degradation and natural attenuation processes in the aquifer. The limited overlap between both approaches demonstrates that each framework captures different fractions of the contaminant spectrum.

These results show that integrating targeted and suspect screening methodologies provides a more complete assessment of groundwater pollution under drought-intensified conditions that enhance the mobility and diversity of ECs. This combined strategy improves the detection of contaminants relevant to current and future

European groundwater quality standards and supports more effective monitoring in vulnerable Mediterranean aquifers.

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3.15.P-Th076 Bridging Polarity Gaps in Groundwater Contaminant Analysis with a Hybrid SPE Approach Combined with Non-Targeted Analysis

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Urban groundwater is increasingly viewed (and even used) as a strategic resource for future drinking-water supplies, especially under growing climate pressures and the need for resilient water systems. Ensuring its long-term safety requires monitoring strategies capable of capturing a broad spectrum of contaminants. While routine surveillance typically focuses on nutrients, heavy metals, and microbiological indicators, this approach overlooks the diverse range of organic micropollutants introduced by human activities. Sources such as wastewater leakage, agricultural inputs, surface water infiltration, or urban runoff contribute complex mixtures of both well-known and emerging organic compounds. Consequently, relying solely on target-based analytical lists provides an incomplete understanding of groundwater quality and may underestimate potential risks.

Non-target analysis (NTA) using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) offers a powerful alternative, enabling the detection of thousands of features without predefined analyte selection. Nevertheless, achieving broad chemical coverage remains challenging due to the diverse physicochemical properties of groundwater contaminants, including their polarity, molecular size, ionisation behaviour, and sorption affinities. A single extraction or chromatographic method often fails to capture the entire polarity range.

To address this limitation, we developed a multi-layered solid-phase extraction (mLSPE) approach that integrates sorbents with complementary retention mechanisms, aiming to maximise the extraction of both polar and non-polar organic compounds within a single workflow. Extracts were analysed using two orthogonal chromatographic modes, hydrophilic interaction chromatography (HILIC) and reversed-phase liquid chromatography (RPLC), coupled to a timsTOF Pro 2 high-resolution mass spectrometer (Bruker Daltonics). This dual-LC strategy expands the detectable chemical space and enhances molecular coverage in NTA workflows.

The combination of multi-layer SPE with complementary LC-HRMS separations significantly widens the analytical window accessible for non-target screening of urban groundwater. This integrated approach supports a more comprehensive characterisation of contamination patterns, provides deeper insight into pollutant sources, and strengthens the overall framework for groundwater protection and risk assessment.

Disclaimer/Disclosure:

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3.15.P-Th077 Advancing Non-Target Screening for Groundwater Monitoring: Combining Reversed Phase and Hydrophilic Interaction Chromatography for Broader Contaminant Coverage

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Groundwater provides nearly half of the world's drinking water, making its protection critical for public health. Organic micropollutants (OMPs)—including pharmaceuticals, pesticides, industrial chemicals, and their transformation products—pose emerging challenges due to their persistence and potential toxicity even at trace concentrations. Traditional monitoring relies on targeted analytical methods optimized for specific compounds, but these approaches may provide an incomplete OMP profile and underestimate risks.

To address this gap, non-target screening (NTS) using liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) is increasingly implemented in monitoring. However, the diversity of OMP physicochemical properties complicates comprehensive detection. Implementing both reversed phase (RP) and hydrophilic interaction liquid (HILIC) chromatography methods will likely improve compound coverage, especially for highly polar compounds ($\log D < 0$).

The Aquaplexus project aims to develop robust LC-HRMS NTS workflows for routine groundwater monitoring in Denmark. We evaluated five LC methods (four RP and one HILIC) coupled to a Bruker timsTOF Pro2 HRMS. Nine groundwater samples were concentrated by vacuum-assisted evaporative concentration (VEC). Pooled samples were spiked post-VEC with 205 OMPs ($\log D_{pH7}$: -16.9 to 7.8).

Method performance was assessed based on number of compounds detected, peak characteristics and intensity, retention time range, and matrix effects. Additionally, data from unspiked samples were analyzed in a non-target workflow to compare chemical fingerprints and OMP identifications across methods. The first results showed significant differences in peak intensities but few differences in OMP coverage between methods. Combining RP and HILIC resulted in a minor increase in OMP detections but a significant increase in sensitivity. Assessments of matrix effects and NTA comparisons will provide a more comprehensive foundation for selecting the best method combination for Danish groundwater monitoring. This work advances NTS implementation for environmental surveillance and informs future monitoring programs aimed at safeguarding drinking water resources.

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3.16.A Integrating Genomics, Metabolomics, and Control Strategies to Address Harmful Algal and Cyanobacterial Blooms

3.16.A.T-01 Mixture Toxicity of Guanitoxin and Organophosphates in Zebrafish: From Developmental and Neurobehavioral Phenotypes to Transcriptomic Responses

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Guanitoxin, a neurotoxin produced by cyanobacteria and the only known naturally occurring organophosphate, poses environmental risks comparable to synthetic organophosphate insecticides. Its effects on aquatic organisms, particularly in combination with other compounds sharing similar mechanisms of action, remain poorly understood. This study evaluated the developmental toxicity of guanitoxin, produced by *Sphaerospermopsis torques-reginae* ITEX-024, alone and in combination with the organophosphate insecticides malathion and trichlorfon in zebrafish (*Danio rerio*) larvae. Embryos were exposed for 120 hours to individual compounds and binary or tertiary mixtures at environmentally relevant concentrations using the Fish Embryo Toxicity test. Multiple endpoints were assessed to evaluate developmental and molecular effects. All compounds exhibited dose-dependent toxicity, with mixtures significantly enhancing both lethal and sublethal effects. The trichlorfon + guanitoxin combination was particularly potent, causing high rates of embryo coagulation, cardiac arrest, hatching failure, and pericardial swelling exceeding 600%. Sublethal effects were observed in all mixtures, including edema, craniofacial malformations, muscle disorganization, and pronounced hypoactivity under dark conditions, with locomotion reduced by ~80% in larvae exposed to guanitoxin + malathion. Transcriptomic profiling revealed downregulation of genes critical for muscle contraction, synaptic signaling, energy metabolism, and oxidative stress. The highest number of differentially expressed genes was observed in the trichlorfon + guanitoxin mixture (1,348 genes), followed by guanitoxin + malathion (513). Synergistic interactions in multiple mixtures indicate that guanitoxin, alone or combined with synthetic organophosphates, exerts severe neuromuscular and developmental toxicity. These findings highlight the ecological risks of pollutant mixtures in freshwater systems affected by agricultural runoff and cyanobacterial blooms, emphasizing the need to integrate mixture effects into environmental monitoring and risk assessment.

Disclaimer/Disclosure:

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3.16.A.T-02 Herbicide Exposure Alters Transcriptomic Responses in a Bloom-Forming Cyanobacterium and Its Chytrid Parasite

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Metolachlor (MET) is a widely used herbicide of the chloroacetanilide family that poses risks to freshwater ecosystems. Beyond its direct toxicity, MET can disrupt ecological interactions such as those between cyanobacteria and their chytrid parasites. Chytrids are zoosporeic fungi ubiquitous in aquatic environments that lethally parasitize phytoplankton, thereby influencing bloom dynamics by delaying or suppressing bloom formation. While previous studies have shown that MET affects cyanobacterial growth and metabolism, as well as chytrid fitness, the molecular mechanisms underlying these effects remain poorly understood. This study investigated the transcriptional responses of the toxigenic cyanobacterium *Planktothrix agardhii* and its obligate chytrid parasite *Rhizophydium megarrhizum* exposed to an environmentally relevant concentration of MET using a dual-transcriptomic approach. MET modulated the expression of genes associated with key physiological processes in both organisms, including photosynthesis in the host, *P. agardhii* and developmental transitions in the parasite *R. megarrhizum*. Furthermore, MET altered host-parasite co-expression patterns, indicating a coordinated molecular response to herbicide exposure. Our findings reveal the molecular basis of MET-induced disruption of cyanobacteria–chytrid interactions, providing new insights into how anthropogenic pollutants can reshape microbial relationships and influence freshwater bloom dynamics.

Disclaimer/Disclosure:

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3.16.A.T-03 Silent Killers on the Riverbed: Episodic Anatoxin Bursts from Benthic Cyanobacterial Mats in Swiss Waters

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Benthic cyanobacteria form cohesive mats on river and lake beds that can remain unnoticed until detached fragments accumulate along recreational shorelines. These mats may contain variably high concentrations of neurotoxic anatoxins and have been linked to rapid dog fatalities after contact with stranded material. In September 2024, several dogs showed lethal neurotoxic symptoms within minutes after bathing and drinking in the River Rhine near Schaffhausen (Switzerland), whereas benthic mats observed at the same reach in March 2025 were not associated with intoxications. This contrast illustrates the episodic and site-specific nature of the risk and the limitations of monitoring strategies that focus mainly on pelagic blooms.

We investigate benthic cyanobacterial mats from river Rhine and selected lakes in northeastern Switzerland using an integrated field and laboratory approach. Mats are sampled as floating and bottom-attached material, and lyophilised biomass is analysed by liquid chromatography–high-resolution tandem mass spectrometry (LC-HRMS/MS) for anatoxins, microcystins and other cyanopeptides, complemented by suspect screening against CyanoMetDB. Parallel DNA analyses (16S rRNA amplicon sequencing and toxin biosynthesis marker genes) provide community composition and the potential for toxin production.

We observe seasonal shifts in both anatoxin load and dominant genera, with *Microcoleus*-dominated mats in late summer exhibiting much higher anatoxin concentrations than *Oscillatoria*-dominated mats the following spring at river Rhine. These findings suggest that acute shoreline risk is strongly strain-dependent and can vary by orders of magnitude over time. By linking environmental conditions, community structure and toxin profiles across sites, this work aims to identify environmental “hot spots” and “hot moments” of anatoxin production and to support more targeted surveillance and timely risk communication for bathing and dog-walking areas.

3.16.A.T-04 Ecophysiological Experiments on *Prymnesium parvum*: Environmental Conditions Favoring Growth

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Invasive and toxic algae threaten ecosystems and public health by spreading rapidly, outcompeting native species, and releasing harmful metabolites. This study examines environmental factors regulating the growth of two harmful genera, *Prymnesium* and *Raphidiopsis*. *Prymnesium parvum* is a toxic, bloom-forming haptophyte that produces ichthyotoxins responsible for major fish kills and food-web disruption while *Raphidiopsis raciborskii* is a filamentous cyanobacterium capable of forming dense, toxin-producing blooms that degrade water quality and endanger aquatic life and human health. Together, these species illustrate the increasing challenges posed by harmful algal blooms in freshwater and coastal systems. Two strains of *P. parvum* (from Poland and Scotland) and one strain of *R. raciborskii* from northeastern Poland were used to assess growth under controlled conditions. Cultures were established through single-cell or single-filament isolation and expanded in climate-controlled chambers. *R. raciborskii* was maintained in WC medium, while *P. parvum* was grown in F/2 medium. Growth was evaluated across a wide range of environmental conditions, including temperatures from 12.5 to 30 °C, conductivities of 750–13,500 µS/cm, pH values of 6.5–8.5, total nitrogen of 1.0–5.0 mg/L, total phosphorus of 0.5–2.5 mg/L, and iron concentrations of 0.1–0.6 mg/L. Experiments were conducted in 100 ml flasks containing 70 ml of medium and 5 ml of culture per strain and were monitored over a 15-day period. Daily, 5 ml subsamples were analyzed for cell abundance and replaced with fresh medium. Although *R. raciborskii* trials have not yet begun, the *P. parvum* experiments show distinct strain-specific responses. The Scottish strain grew best at 13,500 µS/cm, while the Polish strain peaked at 10,500 µS/cm. Both strains performed optimally at pH 6.5–7.0, though maximum cell densities differed. High nitrogen favored the Scottish strain, whereas phosphorus stimulated growth in both; the Scottish strain responded most strongly to the highest concentrations, while 2.5–3.0 mg/L was optimal for the Polish strain. Growth also increased with rising iron levels, with the Polish strain reaching the highest abundances. Temperature experiments for *P. parvum* are ongoing, and identical environmental gradients will be applied to *R. raciborskii* to support the development of effective management strategies. The study also highlights the need to develop strain-specific mitigation strategies.

Disclaimer/Disclosure:

Abstracts have been prepared for *Prymnesium parvum* and *Raphidiopsis raciborskii*. *R. raciborskii* is currently being isolated; if successful, a growth-rate experiment will be conducted. Otherwise, the presentation will focus on two strains of *P. parvum*. Additionally, the temperature experiment for *P. parvum* is ongoing, so its results are not yet included.

3.16.A.T-05 Dual Roles of Cyanobacterial metabolites: As Enzyme Substrates and Inhibitors

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Cyanobacterial blooms release toxic and bioactive secondary metabolites that threaten both ecosystem and human health. Biotransformation plays an important role in determining the environmental fate of these metabolites in surface waters. Research on the biotransformation of cyano-metabolites has focused primarily on microcystins, however, much less is known about other coproduced metabolites such as anabaenopeptins, cyanopeptolins, and microginins. The toxic mode of action of these bioactive metabolites often involves the inhibition of functional enzymes. Yet our understanding of their inhibitory effects on aquatic enzymes involved in biotransformation remains limited. In this study, we investigated the dual roles of cyano-metabolites as enzyme substrates and enzyme inhibitors. Using fractionated cyanobacterial extracts, we examined their reactions with commercial peptidases, the enzyme pool of aquatic biofilms, and a heterologously expressed multicopper oxidase from wastewater activated-sludge communities. Regarding the role as enzyme substrates: carboxypeptidase Y degraded two linear microginins by removing their C-terminal tyrosine residues. The aquatic enzyme pool degraded a wide of cyano-metabolites mainly through peptide-bond hydrolysis, tyrosine oxidation and arginine deamination. Cyano-metabolite containing tyrosine residues were labile when incubated with the multicopper oxidase in the presence of ABTS as a mediator, whereas tyrosine-free congeners remained stable. Regarding the role of enzyme inhibitors: cyanopeptolins inhibited trypsin (IC₅₀ = 53.75 nM), chymotrypsin (IC₅₀ = 2.20 µM) and peptidase activity in aquatic enzyme pool, but not carboxypeptidase Y. Microginins and anabaenopeptins showed mild inhibition towards carboxypeptidase Y and little effects toward the other enzymes. Overall, cyano-metabolites can act both as substrates and inhibitors of environmental relevant enzymes, and the diverse interactions shaping cyano-metabolite fate in surface waters requires further investigation.

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3.16.B Integrating Genomics, Metabolomics, and Control Strategies to Address Harmful Algal and Cyanobacterial Blooms

3.16.B.T-01 Profiling the Metabolomic Response of *Microcystis aeruginosa* PCC7806 to UV-A and UV-B Irradiation Stress

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Understanding the metabolic response of the bloom-forming cyanobacterium *Microcystis aeruginosa* to UV irradiation, a crucial component of sunlight, is essential for assessing ecological impacts and water quality. This study investigates the differential metabolomic profiles of the toxicogenic model strain PCC7806 following exposure to distinct UV-A (320–400 nm) and UV-B (280–320 nm) irradiation treatments.

We employed an integrated untargeted LC-HRMS and a computational pipeline to analyze intracellular metabolites. Cultures were subjected to UV-A (8 h/day for 4 days, sampled after 4 days) or UV-B (15 or 60 min, sampled after 1 and 4 days) and compared to non-irradiated controls. Raw data were processed using *mzmine* for feature detection and precursor-matching against CyanoMetDB, followed by molecular networking and annotations in the Global Natural Products Social Molecular Networking platform (GNPS2) and SIRIUS for in silico annotations. FBMNstats was used for uni- and multivariate statistics.

Statistical evaluation revealed the differential abundance of over 150 annotated features, including cyanotoxins (i.e., MC-LR, [D-Asp3]MC-LR, MC-HiLR), UV-protective mycosporine-like amino acids (i.e., shinorine, porphyra-334, mycosporine-glycine-alanine), and bioactive peptides (aerucyclamides, cyanopeptolins). We observed a marked difference in stress response with UV-A irradiation acting as a stimulant, causing a statistically significant increase in secondary metabolites and overall cell growth (as indicated by higher optical density). Conversely, UV-B induced dose-dependent effects, where short exposure (15 min) resulted in stable or slightly elevated metabolite levels, while prolonged exposure (60 min) caused a significant decrease. These findings provide molecular insights into the complex mechanisms governing *M. aeruginosa* survival and metabolic potential in UV-rich freshwater environments.

3.16.B.T-02 Evaluation of Ultrasound for Cyanobacteria Suppression Using a Field Unit in a Laboratory Anechoic Tank

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Cyanobacteria dominate harmful algal blooms (cyanoHABs) in freshwater sources, including Ohio drinking water reservoirs. Hydrogen peroxide (H₂O₂) is considered an environmentally friendly algacide because it decomposes into water and oxygen and selectively suppresses cyanobacteria. Ultrasound has also been proposed as a non-chemical alternative for cyanoHAB control, with manufacturers claiming it reduces buoyancy and photosynthesis. However, laboratory sound pressures and frequencies often differ from field conditions, complicating translation of results. This study examined whether ultrasound induces sonoporation—temporary openings in cell membranes—and whether this enhances H₂O₂ effectiveness at low concentrations. Experiments were conducted in a 1.2 m cube anechoic tank (1,800 L) lined with acoustic tiles to simulate a reservoir. A commercial ultrasound unit cycled through 24–205 kHz frequencies. Sound pressures were measured with a hydrophone. Cyanobacteria (*Microcystis*, *Anabaena*) were tested at 10⁷ and 10⁸ cells/mL. Sonoporation was assessed using DAPI and SYTOX Green stains quantified by flow cytometry. Synergy tests combined ultrasound with 0, 2, and 4 mg/L H₂O₂. Samples were submerged in Whirlpak bags under controlled light and temperature. Sonoporation experiments lasted up to 4 hours; H₂O₂ treatments were monitored for 48 hours. Algal reduction was measured via cell counts and chlorophyll-a/pheophytin-a fluorescence; residual H₂O₂ was analyzed by UV-Vis spectrophotometry. Ultrasound produced peak sound pressures of 4.3 kPa, below cavitation thresholds, and did not induce sonoporation in *Microcystis* cells. Combining ultrasound with H₂O₂ did not enhance cyanoHAB suppression. At 10⁷ cells/mL, chlorophyll-a was higher with ultrasound after 48 hours, suggesting a protective response to sound, as observed in other bacteria. At 10⁸ cells/mL, no significant differences were found between treatments. Overall, ultrasound did not improve H₂O₂ efficacy and may even promote growth at certain conditions.

Disclaimer/Disclosure:

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3.16.B.T-03 ANTIMICROBIAL RESISTANCE IN CYANOBACTERIA: AN EXPERIMENTAL AND GENOMIC APPROACH

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In 2015, the World Health Organization (WHO) declared antibiotic resistance (AR) as one of the main threats to public health. Therefore, the WHO has promoted a Global Action Plan to combat AR using a One Health approach, which emphasizes the close link between human, animal, and environmental health. In the past decade, it has become clear that the environment influences the spread and transmission of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) through various mechanisms. Cyanobacteria, photosynthetic prokaryotic organisms, are naturally present in almost all terrestrial and aquatic habitats and a growing number of studies highlight some role for cyanobacteria in the spread of AR in freshwater environments. In our study, we tested the susceptibility/resistance of four cyanobacterial strains to environmentally and health-relevant classes of antibiotics at concentration that reproduce the low environmental levels (0.0008 - 0.8 mg/L). We determined the minimum inhibitory concentration (MIC) of two strains of unicellular cyanobacterium *Microcystis aeruginosa* (CCAP1450/6 and CCAP1450/16 from Culture Collection of Algae and Protozoa) and two strains of the filamentous cyanobacterium *Planktothrix rubescens* (F1 and F9 isolated in our laboratory from Flumendosa Lake, Sardinia, Italy), using the microdilution method on 96-well plates. The results showed inter- and intraspecific variability among cyanobacteria with filamentous strains generally more resistant than unicellular ones. Genomic analysis confirmed the MIC tests results: *P. rubescens* strains exhibit antibiotic resistance mechanisms (e.g., the presence of β -lactamase) that explain their greater resistance compared to *M. aeruginosa*, in which no ARGs have been found, demonstrating the genetic susceptibility of both unicellular strains to the tested antibiotics.

Genomic analysis also revealed the presence of mobile genetic elements (plasmids and transposons), suggesting potential mechanisms of horizontal gene transfer responsible for the environmental spread of antibiotic resistance genes.

In conclusion, our results contribute to clarify the potential role for cyanobacteria as reservoirs and vectors of ARGs in aquatic ecosystems.

3.16.B.T-04 Uncovering the cytotoxicity of secondary metabolites from *Microcystis* extracts on two fish cell lines

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Cyanobacterial blooms in freshwater ecosystems have intensified globally due to climate change and eutrophication. *Microcystis* is particularly concerning for producing microcystins (MCs), regulated cyanotoxins. However, other metabolites (e.g., aeruginosins, microginins, microcyclamides) have sublethal to lethal effects, yet remain incompletely characterized and unregulated.

This study screened 14 *Microcystis* strains with distinct metabolite profiles for ecotoxicity using in vitro fish cell lines: rainbow trout liver-waterloo 1 (RTL-W1) and common carp leukocyte (CLC). Flow cytometry assessed cell mortality and growth after 24 h and 48 h. A Weight-of-Evidence-based method integrated toxicity data into a ToxHQ index normalized between reference, methanol control, and 3,4-dichloroaniline (3,4-DCA), a cytotoxicity positive control (OECD-recommended positive control). Cell cycle and energy metabolism (glycolysis and mitochondrial stress) were analyzed by flow cytometry and Seahorse. Metabolomic analyses were performed by LC-MSMS.

Extracts induced cell mortality ranging from 5% to 47% and growth inhibition from 0.41 to 0 day⁻¹ compared to the 89% of cell mortality and 0 day⁻¹ of growth inhibition in the positive control (3,4-DCA), particularly the CLC cell line. PMC 815.12 extract ranked in “moderate” toxicity associated with a higher ToxHQ Index than PMC 570.08 (36.7% vs. 20.9%), which also produced emerging cyanopeptides, including cyclamides and cyanopeptolins. Five additional extracts showed slight effects (ToxHQ Index 8 to 13%), producing MCs and/or emerging cyanopeptides (aeruginosins, cyanopeptolins, cyclamides, microguanidines), alongside non-peptide and unknown metabolites. These findings raise questions about which metabolites – and/or variants- drive these effects and suggest potential molecular interactions. Sublethal effects were investigated, including cell cycle analysis and energy metabolism. Some extracts (cyclamides, microginins, microcystbiopterins, cyanopeptolins, aeruginosins) inhibiting the growth rate, affected cell proportions in G1, S, and G2 phases. A few extracts were tested for metabolism (glycolysis and mitochondrial stress) showed no significant effect. Effect-Directed Analysis will fractionate toxic extracts to identify causative metabolites. Additional sublethal endpoints would refine toxicity assessment and improve understanding of mixture effects on aquatic organisms

3.16.B.T-05 Bioprospecting Microcystis-Dominated Toxic Biomass For Valuable Compounds And Biopesticidal Activity

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Climate change and eutrophication are intensifying toxic Microcystis blooms, posing threats to freshwater ecosystems and public health. While mechanical harvesting reduces acute risks, sustainable management requires economic valorization of the collected biomass in line with circular economy principles. This study aimed to identify valuable compounds in Microcystis-dominated biomass and assess the potential of its extracts as bio-based pesticides against plant pathogens.

Cyanobacterial biomass was harvested from the Kaunas Reservoir, Lithuania (2020–2024), using AS-L/AS-LAND prototypes. Freeze-dried samples were analyzed for macromolecular content (proteins, pigments, carbohydrates, lipids). Individual compounds were identified via LC-qTOF in 70% methanol extracts. The antagonistic activity of four extract types (water, methanol, acetone–ethanol, phycocyanin) was tested against the oomycete *Phytophthora cactorum* and seven fungal pathogens (including *Fusarium*, *Botrytis*, and *Alternaria* spp.). Pathogen growth on extract-amended media was assessed over 14 days.

Proteins were the most abundant component in the biomass (45.9 ± 9.5 mg/g), significantly exceeding lipids and carbohydrates. LC-qTOF analysis revealed a diversity of bioactive non-ribosomal peptides, including anabaenopeptins, cyanopeptolins, microginins, and oscillamides. Methanol extracts were dominated by microcystins (21.4–47.1%) and pigments (13.7–56.4%). In bioassays, aqueous extracts were ineffective or stimulatory to pathogen growth. Conversely, methanol extracts demonstrated strong inhibitory activity, reducing *Fusarium culmorum* growth by up to 22.5% and *Phytophthora cactorum* by up to 44.6% after one week. Morphological changes were also observed in *Alternaria alternata* and *Botrytis cinerea*.

Harvested Microcystis biomass is a rich source of proteins and bioactive peptides. While aqueous extracts showed limited utility, methanol extracts effectively suppressed specific plant pathogens. These findings highlight the potential for valorizing toxic cyanobacterial blooms into biopesticides, transforming an environmental hazard into a valuable resource.

Disclaimer/Disclosure:

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3.16.P Integrating Genomics, Metabolomics, and Control Strategies to Address Harmful Algal and Cyanobacterial Blooms

3.16.P-Tu284 Mass Spectrometry Query Language (MassQL) - Driven Pipeline for Comprehensive Detection of Structurally Diverse Cyanopeptides in Environmental Datasets.

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Cyanobacteria produce a structurally diverse array of secondary metabolites, including potent toxins and bioactive molecules. Rapid and accurate detection of these compounds in complex environmental and biological matrices using mass spectrometry (MS) is essential for environmental hazard assessment and natural product discovery. However, the high structural variability within major cyanopeptide classes (e.g., microcystins, anabaenopeptins, microginins, cyanopeptolins) often leads to incomplete detection when relying solely on matching with publicly available spectral libraries, which are limited and incomplete.

To address this gap, we have developed a computational screening approach which begins with feature-based molecular networking (FBMN) to map and categorize known and potential cyanopeptide families within the MS datasets. While FBMN successfully reveals clusters of related compounds, this project develops class-specific mass spectrometry query language (MassQL) queries, deployed within the Global Natural Products Social Molecular Networking (GNPS2) ecosystem for sharing and community curation of mass spectrometry data, to provide a robust, complementary, and transferable methodology for comprehensive screening across diverse MS2 datasets.

Our approach centers on developing class-specific MassQL queries by systematically identifying and incorporating the minimal set of required and excluded MS2 fragmentation patterns (diagnostic fragment ions and neutral losses). The core objective is to simultaneously minimize Type A errors (false negatives), ensuring near-comprehensive coverage of structural analogs, and Type B errors (false positives), maximizing specificity against unrelated compounds.

The resulting MassQL queries are designed for direct implementation across both proprietary in-house libraries and vast publicly accessible metabolomics repositories. This work demonstrates the power of a standardized, pattern-matching query language to transform high-throughput screening, providing the environmental and public health communities with a much-needed tool for rapid and comprehensive detection of structurally diverse cyanopeptides.

Disclaimer/Disclosure:

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3.16.P-Tu285 Chemodiversity of Microginins and Aeruginosins in Greek Freshwater Bodies: Elucidation of Novel Linear Peptides

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Cyanobacteria form Harmful Algal Blooms (HABs) that are occurring with increasing frequency and intensity in recent years, often lasting for extended periods as a consequence of climate change. The production of cyanotoxins such as microcystins during HABs poses a major threat to freshwater ecosystems and public health. Along with well-known cyanotoxins, cyanobacteria produce other bioactive metabolites which are often overlooked.

This study focuses on investigating the presence and chemodiversity of the bioactive linear cyanopeptides belonging to the microginin and aeruginosin classes in freshwater bodies of Greece. Samples were collected during HAB events and analyzed using liquid chromatography – high-resolution mass spectrometry (LC-HRMS) with data dependent acquisition (DDA). Acquired data were processed using a comprehensive pipeline of open-access software and platforms, including mzmine for feature detection and searching to CyanoMetDB compound database, MetFrag for in-silico fragmentation of possible structural candidates, GNPS for feature-based molecular networking and spectral library matching as well as SIRIUS for formula prediction and structure annotation. Feature-based molecular networking successfully revealed the molecular networks of microginins and aeruginosins. This advanced data analysis approach enabled the elucidation of numerous known and new congeners of these linear peptides, significantly contributing to the understanding of the metabolic potential of bloom-forming cyanobacteria. Results reveal a previously underestimated chemodiversity within the linear peptide profile of cyanobacterial blooms. Furthermore, this approach was proven efficient for the characterization of other overlooked metabolites in complex natural product mixtures particularly when hindered by the absence of reference standards and open-access reference fragmentation spectra.

3.16.P-Tu286 Grazer pressure and its influence on growth and toxin dynamics in harmful algae: insights from copepod interactions

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Harmful algal blooms (HABs) can pose significant ecological and socio-economic risks by producing toxic metabolites that accumulate in food webs, as such impacting fisheries, aquaculture, tourism, and human health. Anthropogenic pressures, including greenhouse gas emissions and nutrient enrichment, have intensified climate change and eutrophication, altering key abiotic factors such as water temperature, pH, salinity, and nutrient ratios. These changes have been demonstrated to influence HAB dynamics, and were identified as critical drivers of growth and toxin production. Previous studies suggest HAB toxins deter grazing from predators, but the reciprocal effects of grazers on HAB physiology remain underexplored. Current evidence is limited but suggests that certain chemical cues released by grazers - such as copepodamides excreted by copepods - can trigger toxin production in harmful algal species like *Alexandrium* and *Pseudo-nitzschia*. The mechanisms driving this phenomenon remain poorly understood, as does its consistency across diverse algal species and grazer-derived chemical signals. It is also unknown whether these interactions are modulated by environmental conditions. Furthermore, broader physiological responses and comparative effects among other grazer groups (e.g., bivalves) are poorly understood.

To address these knowledge gaps, we examined both direct and indirect effects of grazer pressure on HAB growth and toxicity. Two HAB species - the dinoflagellate *Alexandrium minutum* (a saxitoxin producer) and the estuarine cyanobacterium *Microcystis aeruginosa* (a microcystin producer) - were exposed to (1) live copepods, (2) copepod extracts, and (3) copepod culture medium filtrates (potentially containing chemical cues). We quantified growth rates, intracellular and extracellular toxin concentrations, and photosynthetic pigment profiles as indicators of algal physiological status. Copepod grazing rates were also assessed. Comparative experiments included three copepod species *Nitokra spinipes*, *Acartia tonsa*, and *Temora longicornis*, the dominant North Sea species. This study provides novel insights into grazer-HAB interactions and advances our understanding of feedback mechanisms that influence bloom dynamics under changing environmental conditions.

3.16.P-Tu287 Cyanobacteria Blooms in Shallow Urban Ponds: Insights from Seasonal and Bi-Monthly Sampling in 20 Ponds in Brussels (Belgium)

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Small, shallow urban lakes provide important ecosystem services, such as biodiversity support and recreational opportunities, which are closely linked to phytoplankton biomass and community composition. In several Brussels ponds (Belgium), potentially harmful cyanobacteria blooms (cHABs) are recurrently observed. This study aims to describe and compare cyanobacteria dynamics across twenty urban ponds, addressing the challenge of identifying appropriate methodological strategies to efficiently capture changes in phytoplankton assemblages. We combined a single year of seasonal sampling across all twenty ponds with more than two years of (bi)monthly sampling in a subset of four ponds. Phytoplankton dynamics were assessed using multiple complementary approaches: chlorophyll *a* measurement as a proxy for total phytoplankton biomass, chemotaxonomic pigment analysis to estimate major phytoplankton groups, microscopy for cross validation of cyanobacteria identification, and 16S ribosomal DNA (rDNA) sequencing with cyanobacteria-specific primers. Seasonal sampling was useful for identifying ponds with low biomass and low risk of cHABs, but proved insufficient to capture cyanobacteria dynamics. Chlorophyll *a* concentration alone did not reliably indicate cyanobacteria biomass, as high values frequently reflected blooms of other phytoplankton groups, particularly cryptophytes and chlorophytes. Chemotaxonomic analysis efficiently captured cyanobacteria evolution, revealing elevated biomass especially during summer, but lacked fine-scale taxonomic resolution. Microscopy offered direct and timely identification but was constrained by its labor intensive and operator dependent nature. In contrast, 16S rDNA sequencing provided finer taxonomic resolution, including the detection of picocyanobacteria not visible under standard microscopy, yet remains inadequate for routine monitoring. Results showed that cHABs were mainly dominated by the genera *Dolichospermum*, *Microcystis*, and *Aphanizomenon*, all associated with potentially toxic strains. Overall, our findings highlight the need for combining multiple monitoring approaches to assess cyanobacteria dynamics in urban ponds.

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3.16.P-Tu288 Diversity, Toxicity, and Chemical Characterization of Marine Benthic Cyanobacterial Mats from Florida Coastal Ecosystems

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Marine cyanobacteria are diverse and widespread across estuaries and bays to open oceans, producing a wide range of secondary metabolites including cyanotoxins. Benthic cyanobacterial mats (BCMs) are increasingly reported in marine environments, partly driven by nutrient loading and climate change. Along Florida coasts, BCMs have proliferated across mangroves, seagrass beds, and coral ecosystems, demonstrating the urgency in understanding the diversity, physiology, and toxicity of this growing environmental health hazard. Between 2022 and 2024, BCMs were sampled to investigate their diversity and toxin production. Environmental samples underwent eDNA extraction with amplification of 16S rRNA for bacteria including cyanobacteria, and 18S rRNA for associated eukaryotes. Individual strains representing novel and published species were isolated and cultured for morpho-molecular characterization. Metagenomes of select field samples and individual strains, along with nutrient and metal analyses were investigated. Sequencing revealed that BCMs are diverse communities primarily composed of large filamentous cyanobacteria including *Dapis*, *Okeania*, *Lyngbya*, *Sirenicipillaria*, and *Moorena* while also acting as reservoirs for several bloom-forming dinoflagellates including *Amphidinium* and *Alexandrium*. Toxin profiles were determined using complementary analytical approaches. Targeted screening by LC-MS/MS focused on ~40 characterized cyanotoxins including paralytic shellfish toxins (PSTs), anatoxins, cylindrospermopsins, microcystins, and lyngbyatoxins. Samples showed the presence of anatoxin-a, dihydroanatoxin-a, cylindrospermopsins, and microcystins. A dmMC-RR analogue was detected in two *Moorena* species. For lyngbyatoxins, a specific analytical method was developed in-house based on a contaminated sample from New Zealand, enabling their identification and quantification. Results indicate that Florida's benthic cyanobacterial mats represent complex, diverse communities producing multiple known and potentially novel toxins and secondary compounds. Further assessment is needed to fully characterize their cyanobacterial and eukaryotic community structure, toxin production capabilities, and ecological drivers, emphasizing the importance of continued monitoring of these proliferating marine cyanobacteria.

3.16.P-Tu289 Monitoring Harmful Algae Blooms In Ecuador: Challenges And Search For Solutions

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Cyanobacterial blooms and its toxin production represent a growing environmental and public health concern in Ecuador. Conventional toxin detection methods such as HPLC and ELISA are expensive and logistically challenging in this region. We use environmental DNA (eDNA) as alternative approaches for monitoring potential (toxic) bloom events. A longitudinal and temporal analysis of water samples between 2022-2025 was performed, targeting five strategic locations along the Daule's river basin; and seven water reservoirs. Using eDNA, three microcystin synthesis genes (*mcyA*, *mcyB*, and *mcyE*) and two cylindrospermopsin synthesis genes (*cyrA* and *cyrC*) were monitored. Also, cyanobacteria-specific primers were employed to demonstrate their presence/absence. We detected distinct spatiotemporal distribution patterns of the cyanotoxin genes: microcystin-related genes were detected more frequently lotic sites, while cylindrospermopsin-genes showed higher prevalence in lentic water bodies. These results indicate a habitat preference: microcystin-producing species predominantly occur in riverine systems, while cylindrospermopsin-producing species dominate in reservoir environments. The observed distribution patterns suggest that cyanobacterial species composition is significantly influenced by water body type and associated environmental factors. Furthermore, as microcystin standards are absent in Ecuador, we focused on the development of a biosensor using the bacteria *Sphingosinicella microcystinivorans*. Several strategies were elaborated consisting of transforming *S. microcystinivorans* on the one hand and using its microcystin degradation gene cluster for

transforming *E. coli* on the other hand.

This study demonstrated molecular technologies as sensitive methods for early detection and monitoring of (harmful) algal blooms, presenting a viable alternative to traditional analytical approaches in resource-constrained environments.

3.16.P-Tu290 Non-Targeted Analysis Based on High-Resolution Mass Spectrometry for the Simultaneous Detection of Cyanotoxins and Synthetic Emerging Pollutants in Water Reservoirs

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Water resource depletion and increasing drought frequency are critically elevating the need for systematic quality monitoring of drinking water sources. These environmental stressors often concentrate emerging contaminants, leading to complex mixtures where natural toxins converge with anthropogenic chemicals. In this study, we applied a Non-Targeted Analysis (NTS) workflow to investigate its capability for early detection of natural and synthetic pollutants at trace levels in two key reservoirs supplying the Attica region: Lake Yliki and Lake Marathonas.

Samples collected from diverse locations (water-intake towers, pumping stations, and stream inflows) were analyzed using Liquid Chromatography-Quadrupole Time-of-Flight (LC-qTOF-MS) utilizing both Data-Dependent (DDA) and Data-Independent Acquisition (DIA). The computational pipeline included data preprocessing in MZmine, Feature-Based Molecular Networking (FBMN) on the GNPS platform, and downstream multivariate statistical analysis.

The analysis revealed a co-occurrence of biogenic compounds (cyanotoxins, cyanopeptides) alongside anthropogenic pollutants (pesticides, pharmaceuticals). However, it is important to note that the concentrations of these detected compounds were extremely low (ng/L range). Consequently, the analysis provides solid proof that raw water quality is suitable for drinking water production following standard treatment processes, ensuring that concentrations of pesticides, pharmaceuticals, and cyanotoxins remain well below the established parametric values in accordance with the EU Drinking Water Directive (2020/2184) and World Health Organization (WHO) guidelines.

The critical advantage of this HRMS-based methodology is the capacity to monitor a wide range of natural and synthetic compounds simultaneously, starting from very low levels (ng/L). Unlike traditional targeted monitoring, this approach captures the full spectrum of the chemical cocktail, serving as a powerful tool for early-warning management and environmental forensics, allowing for timely interventions. This high-sensitivity screening enables a proactive system to safeguard water quality, identifying potential contamination pathways—such as agricultural runoff or wastewater inputs—before they pose a significant risk to public health.

3.17.A Integrating Polymer Analytics, Plastic Additives, and Micro/Nanoplastics for Risk Assessment and Regulation

3.17.A.T-01 Rubber additives are bioaccessible and rapidly released from respirable particulate matter in the lungs

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Airborne tire-wear particles generated during driving are ubiquitously present in urban particulate matter and are inhaled by urban residents. These particles contain several rubber additives and their transformation products (rubber-derived compounds, RDCs), many of which are of toxicologic concern to humans. However, the extent to which inhalation of tire-wear particles contributes to RDC exposure depends on RDC bioaccessibility, i.e., whether they are released from particles before particles are expelled from the lungs or destroyed. In this study, we isolated respirable particulate matter (aerodynamic diameter < 4.5 µm) and conducted leaching experiments with simulated epithelial lung fluid and phagolysosomal lung fluid to simulate conditions on the lung surface, and following engulfment by phagocyte cells. RDCs with a wide range of physicochemical properties including hydrophobicity (log D from 0.9 to 4.9), charge state (neutral and positive), and compound stability were bioaccessible in the lungs, although the total bioaccessible fraction varied between compounds, and between simulated lung environments. Bioaccessibility in lung fluids was higher than reported in other aqueous environments, due to the unique composition of lung fluids (high organic content and presence of pulmonary surfactants which enhanced leaching especially for hydrophobic RDCs), as well as the inherently small size of respirable particulate matter. Such fine particles have a low intraparticle diffusion distance, and a high surface area to volume ratio contributing to RDC release. For all bioaccessible RDCs, release kinetics were rapid, reaching equilibrium concentrations in lung fluids within ten minutes. These rapid kinetics were due to small particle size, and high local solid to liquid ratios within the lungs. Fast leaching kinetics imply that RDCs will

be released during rubber particles pulmonary residence time. Inhalation of rubber particles is therefore an important source of RDC exposure.

3.17.A.T-02 A Fingerprint Analysis Approach Towards Identification and Quantification of Additives in Plastics

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Plastics development used to be all about creating new base polymers. Now, the focus has shifted towards creating new formulations, i.e., combinations of base polymers and functional additives. These additives are crucial for adjusting the properties of plastics, such as resistance to degradation, flame retardancy or flexibility. Given legal requirements, modern formulations also often mandatorily contain recycled plastics, increasing the overall need for those. Preferentially, plastics for recycling are sourced from applications where low additive contents are needed, such as packaging waste, to minimize issues with residual additives.

For example, the regulations regarding flame retardants (FR) have become increasingly strict in recent years and plastics being now recycled often still contain formerly allowed but now highly regulated FR. Issues such as this prevent the recycling of many of the available plastics, with electronic waste being a particularly prominent case where lots of material would be available but recycling efforts are severely hampered by concerns regarding additive content. Increasing the use of these raw material streams in recycling will require suitable analytical approaches for fingerprinting additives in plastics at the recycling site.

While established methods, typically based on chromatography/mass spectrometry exist, these methods suffer from high cost, long analysis times and require a suitable lab. In comparison, ion mobility spectrometry (IMS) offers a highly promising alternative for determining the additive profiles of recycled plastics. It provides high sensitivity, short analysis times, portability, and relatively low costs (both in terms of purchase and operation). Furthermore, it has already demonstrated its immense potential in many areas, particularly in security checks (e.g., explosives detection at airports). Applications such as in environmental analysis further underscore the high sensitivity and specificity of the method in mobile applications. And the general applicability regarding additives, particularly FR, was similarly shown recently. Still, there have been no applications of IMS established with regards to recycled plastics, yet.

The presentation will outline an IMS-based approach towards on-site fingerprinting of additives in recycled polymers. It will detail the necessary steps in method development, foreseen challenges and mitigating approaches as well as initial results.

3.17.A.T-03 Fate of industrial polypropylene pellets in the environment: influence of additives on the release of degradation by-products

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Plastic pollution encompasses a wide range of emerging contaminants, including additives, microplastics, nanoplastics, as well as volatile and soluble species produced during polymer degradation. Although these compounds are now found in aquatic, terrestrial, and biological environments, their contribution to plastic pollution remains widely underestimated and insufficiently characterised. Additives, incorporated to facilitate processing, improve material properties, or extend durability, can leach during use and markedly influence degradation pathways and kinetics. Yet, environmental samples do not allow the initial composition or true exposure conditions of plastics to be determined, making controlled laboratory studies essential to link formulation, ageing mechanisms, and the nature of emitted by-products.

In this work, polypropylene (PP) pellets containing controlled amounts of additives commonly used in commercial plastics were subjected to accelerated ageing protocol combining UV exposure and moderate mechanical stress. Their behaviour was compared with that of additive-free pellets. Mass loss, surface oxidation, and changes in crystallinity were monitored during UV ageing. Volatile by-products were inferred from gravimetric measurements, while particulate and soluble degradation products were quantified using morphometric analysis and total organic carbon measurements in the aqueous phase.

Our results show that polypropylene degradation generates volatile, soluble, and solid by-products regardless of formulation. Additives do not suppress the formation of these species but significantly delay their appearance.

While volatile and soluble compounds are ultimately produced at levels similar to those of neat PP, the generation of microplastics is much more strongly inhibited. Additives shift the degradation mechanism towards surface-limited oxidation and delamination, reducing overall fragmentation favouring the realisation of small

uniform particles. These findings underline the key role of formulation in the environmental ageing of polyolefins and highlight the importance of considering additive effects in future plastic design strategies and pollution assessment frameworks.

3.17.A.T-04 Multi-class Organic Contaminants in Brain and Muscle of Mallorcan Fish

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Plastic additives and personal care product chemicals are widely used in consumer products and can exhibit toxic effects, raising concerns for environmental and human health. Despite growing awareness, information on their tissue-specific distribution and bioaccumulation in wild marine fish remains limited. This study investigated the occurrence and distribution of a broad range of plastic additives, bisphenols, UV-filters, and synthetic musk fragrances in brain and muscle tissues of two Mediterranean fish species commonly consumed by humans: European hake (*Merluccius merluccius*, n = 18) and striped red mullet (*Mullus surmulletus*, n = 8), collected off the coast of Mallorca (Spain).

Concentrations of 20 organophosphate esters (OPEs), 12 phthalates (PAEs), 11 non-phthalate plasticizers (NPPs), 4 bisphenols (BPs), 2 UV-filters, and 1 musk fragrance were quantified using liquid chromatography–high resolution mass spectrometry (LC-HRMS). Results were lipid-normalized to compare tissue distribution between brain and muscle.

PAEs were the predominant compounds, with maximum concentrations reaching 3.82×10^2 ng/g lipid weight in mullet brains and 1.38×10^2 ng/g lipid weight in hake brains. Detection frequencies differed markedly between tissues. Brain tissues showed high detection of DIPA, DEHP, and TCIPP, while muscle tissues were dominated by tonalide, DiBP/DNBP, DEHP, and high-molecular-weight phthalates. Overall, concentrations were generally higher in brain than muscle for compounds detected in both tissues, though clear species-specific trends were observed. Notably, bisphenol A, TPHP, and DINCH were exclusively detected in brain tissue, whereas bisphenol S, octocrylene, TCEP, and DEHP were mainly associated with muscle.

These findings demonstrate compound- and tissue-specific accumulation patterns, with brain tissues accumulating potentially neurotoxic chemicals and muscle tissues representing a relevant pathway for human exposure through seafood consumption. Accounting for tissue-specific distribution is essential for accurate environmental and human health risk assessments.

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3.17.B Integrating Polymer Analytics, Plastic Additives, and Micro/Nanoplastics for Risk Assessment and Regulation

3.17.B.T-01 Applying Effect-Based Testing to Develop Safer Polymers: Case Study with Flame-Retardant, Reprocessable Vitrimers

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The transition toward Safe and Sustainable by Design (SSbD) materials requires early integration of hazard assessment alongside the analysis of functional performance to support material optimization. For novel or

highly engineered polymers, conventional chemical analyses and risk-based exposure assessments may not capture complex or unexpected biological effects arising from leaching substances such as additives, degradation products, or processing-related by-products. Effect-based bioassays offer a valuable alternative strategy as they directly measure biological responses to mixtures of often unknown chemicals, enabling more comprehensive evaluation of potential human and ecotoxicological hazards at early innovation stages. Here, we present a case study on the aquatic toxicity of a newly developed vitrimer material that incorporates dynamic covalent bonds within highly crosslinked networks. This design provides mechanical strength, reprocessability, and inherent flame retardancy, all of which are essential for both high performance and circularity. Phosphorus-containing polymers are particularly promising, since phosphorus contributes to flame retardancy while also establishing dynamic bond exchange. Unlike traditional thermosets where phosphorus is added as an external additive that can increase leaching risk and compromise mechanical properties, our approach aims to embed phosphorus directly into the polymer backbone to provide inherent flame retardancy and reprocessability. Early toxicity assessment is essential to ensure material safety and to guide iterative improvements. To address this need, we applied a fish cell based acute toxicity assay (OECD Test Guideline 249), miniaturized from a 24-well to a 96-well format to increase throughput while maintaining sensitivity, cell viability, and reproducibility. A set of monomers, polymers, and material leachates was evaluated to systematically investigate the influence of formulation, curing, and reprocessing cycles on aquatic toxicity. Combined with material performance data, these effect-based toxicity results supported iterative material optimization, thus showing that integrating bioassays into early research and development can complement or in some cases even replace conventional chemical characterization and help advance SSbD material development.

3.17.B.T-02 Plastic Product-Specific Degradation Pathways and Quantification of Release Species: Towards Systematic Assessments for Material Comparison and Harmonization

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During their use phase, polymer-based products (including paints and textiles) age, fragment and release different materials at rates that vary by many orders of magnitude, depending on the exposure scenario and on the material composition. Consequently, fragmentation and degradation products are released, such as microfibers and -particles, fillers, additives, and/or low-molecular-weight organic species. The rates and profiles of these releases vary significantly depending on the material composition and exposure scenario. Comprehensive assessment of these emissions is essential, not only to quantify solid fragments (e.g., micro- and nanoplastics), but also to capture dissolved degradation products and additives.

To address current analytical limitations – especially in distinguishing different types of release species – novel workflows and analytical protocols have been developed for the cases of paints and textiles. For textiles, full-scale washing machine tests with in-line filtration and Raman microscopy (>50 µm) were combined with batch abrasion simulations. Analytical ultracentrifugation (AUC) and thermogravimetric analysis (TGA) enabled the differentiation between various release species.

Similarly, for paints and coatings, artificial weathering and mechanical stress in the presence of water were applied to facilitate the release of fragments and degradation products. A unified protocol employing Pyrolysis Gas Chromatography Mass Spectrometry (pyr-GCMS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and dissolved organic carbon (DOC) measurements allowed for the quantification of dissolved degradation species and additives, solid polymers, and inorganic or composite particles. These methodological advances enable a holistic and comparative assessment of micro- and nanoplastic releases from polymer-based products. The differentiation and quantification of different types of release species is still challenging and requires the use of several analytical techniques in parallel. The resulting insights provide a foundation for the targeted development of new materials with minimized environmental release profiles.

3.17.B.T-03 Development, Validation, and Application of a Method for the Quantification of Micro- and Nanoplastics in Human Bile

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Micro- and nanoplastics (MNPs) have recently been identified and quantified in human tissues and fluids, raising concerns about potential health implications and highlighting the need for reliable biomonitoring methods. Human bile is a relevant but largely unexplored matrix for assessing internal exposure, as it is a key medium for the excretion of exogenous substances entering the body via ingestion. Its complex composition, together with the difficulty of preventing external plastic contamination during clinical handling, has limited its

use in MNP research. This study aims to address these challenges by developing and validating a method based on pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) for the quantitative determination of six common polymers, namely polymethyl methacrylate (PMMA), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS) and polyethylene (PE), in human bile. In addition to method development, the study also aims to assess the suitability of routinely collected clinical material for MNP biomonitoring. Archived bile samples stored under standard clinical conditions will be evaluated alongside bile collected and processed under strictly plastic-free protocols. This comparison will allow us to identify potential contamination sources associated with common storage conditions and to determine whether existing biobank materials can be reliably used for retrospective analysis of MNP exposure. Bile samples collected during liver transplantation procedures were processed using plastic-free protocols and rigorous QA/QC procedures. Method validation was performed by evaluating all relevant analytical figures of merit, confirming the robustness and suitability of the approach for quantitative MNP analysis in bile. The validated method was applied to an initial set of human bile samples, with PVC identified as the most frequently detected polymer. These findings demonstrate the applicability and potential of the method, which will be extended to a larger dataset and archived clinical material to support future evaluations of internal exposure and the suitability of existing clinical storage conditions for retrospective MNP biomonitoring.

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3.17.B.T-04 So You've Eaten a Microplastic – Where Does it Go? The First Human Microdosing Study

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Human exposure to microplastics is increasingly recognised as a global concern, yet a human risk assessment remains challenging. This is in part due to the lack of biokinetic data on the adsorption, distribution, metabolism and excretion (ADME) of microplastics in the human body. Current evidence for the absorption of microplastic particles upon ingestion is based on in vitro and in vivo rodent studies. The biological relevance of this data is, however, questionable due to the simplicity of the models and potential interspecies differences. In this study, 1 μ m 14C-polystyrene particles and Accelerator Mass spectrometry (AMS) were used to perform the world's first human microdosing study, filling a major data gap by providing biokinetic data on microplastics in the human body.

In this work, we use 14C-labelled polystyrene - which accurately mimics the chemical and physical properties of unlabelled polystyrene - to perform the first human microdosing study. Our approach utilizes a state-of-the-art Accelerator Mass Spectrometer (AMS) which is able to detect trace-levels of 14C. This innovative approach allows detection of PS with high sensitivity in complex matrix samples upon exposure to very low doses, without altering particle behaviour. This overcomes the limitations of conventional spectroscopic or mass-based methods that require fluorescently-labelled or metal-doped particles.

Six healthy normal weight volunteers between 21 and 36 years of age (three males and three females) received a single oral microdose (100 μ g) of 14C-labelled polystyrene incorporated in yoghurt. Blood, urine, and feces samples were collected over a five day period to characterise the absorption and excretion rate of polystyrene microplastics. The clinical trial was successfully performed and preliminary AMS results indicate the presence of 14C in the blood, the quantification and interpretation of which is currently ongoing, along with analysis of urine and feces.

This first-of-its-kind experiment represents a large step towards facilitating a human risk assessment of microplastics by delivering essential, robust biokinetic data in vivo in humans. The methodology - combining isotopic labelling with AMS - also offers a framework for future research as it can be adapted for many polymer types, morphologies and particle sizes.

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3.17.P Integrating Polymer Analytics, Plastic Additives, and Micro/Nanoplastics for Risk Assessment and Regulation

3.17.P-Tu291 Evaluating microplastic particles as vectors of exposure for plastic additive chemicals using a food web model

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Micro- and nanoplastic particles (MNPs) represent potential hazards for humans and wildlife, including as vectors for chemical exposure (e.g. plastic additives and pollutants sorbed from the surrounding environment). The leaching of chemicals from MNPs has been identified as a potential exposure pathway but the relative magnitude of this pathway under environmentally relevant conditions remains unclear. Here, we describe a modification of the ACC-HUMANSTEADY bioaccumulation model to include dietary exposure to MNPs containing either accumulated chemicals from the surrounding environment or embedded plastic additive chemicals (PACs). Chemical transfer to humans and wildlife is described using two-film resistance concepts assuming spheroidal or cylindrical particles of different sizes. The relative contribution of MNPs and environmental media to the estimated daily chemical intake in humans was assessed in various exposure scenarios, for a range of hypothetical chemicals with varying octanol-water and air-water partition coefficients (KOW and KAW, respectively; i.e. $0 < \log KOW < 8$ and $-5 < \log KAW < 3$). Results imply that MNPs could act as sources of exposure to chemical additives when the ingestion rate of 1 μm MNPs is $>10 \text{ mg d}^{-1}$, and the concentration of hydrophobic plastic additive is $>5\% \text{ wt wt}^{-1}$. The contribution made by MNPs as vectors of exposure decreased with increasing particle size and decreasing ingestion rates of MNPs. Human health risks were evaluated for four specific PACs to illustrate the application of the model for risk assessment. Risks were negligible when the ingestion rate of MNPs was $<100 \mu\text{g d}^{-1}$. Uncertainties are high regarding the characterization and quantification of ingestion of MNPs by humans and wildlife, including particle sizes and polymer composition, as well as on the presence of PACs in MNPs. These data gaps need to be addressed if the issue of MNPs as vectors of chemical exposure is to be fully understood. The work illustrates that mechanistic models, which account for all major exposure pathways, can be used to identify the importance of different exposure pathways, help prioritize research needs and support decision making.

3.17.P-Tu292 Understanding Human Exposure to Plastic Additives – A Systematic Review Protocol

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Plastic additives include a wide variety of chemicals which are added to plastics to confer them specific properties such as flexibility, resistance, and toughness/softness. With over 16 thousand plastic additives in use, there are three classes of these chemicals that are receiving growing international attention given their widespread use and detection in the environment: phthalates (PAEs), organophosphate esters (OPEs) and non-phthalate plasticizers (NPPs). Several PAEs and OPEs are associated with adverse effects, such as endocrine disruption, immunotoxicity and carcinogenic effects. For NPPs, which are often used as alternatives to PAEs, toxicological information is still lacking. Nonetheless, some recent studies are showing that these compounds might also have harmful effects in neurodevelopment and on the endocrine system. Due to these health concerns, the number of studies investigating human exposure to these chemicals has been growing in recent years, highlighting that humans can be exposed to PAEs, OPEs and NPPs through diet, dust, air, and consumer products. However, a critical question remains: which is the major route of exposure to these chemicals? To address this question, we designed a systematic review protocol to systematically find and select peer-reviewed literature reporting quantitative data for one or more exposure pathways to PAEs, OPEs, and NPPs. The first step of the protocol consists of searching literature data in various databases (PubMed, Science Direct, Scopus, Web Of Science, Wiley Online Library) and one literature search engine (Google Scholar) using specific keywords for PAEs, OPEs and NPPs in combination with keywords for different exposure pathways (diet, dust, air inhalation, dermal uptake). Duplicates are removed, and the second step consists of a screening based on title and abstract. During this step, each publication is reviewed by at least two reviewers with defined exclusion

criteria to remove all publications without human exposure data on selected plastic additives. The final step consists of reviewing the publications in full text and selecting those for data extraction. In this step, only publications with quantitative estimates of at least one human exposure pathway are included. This systematic review protocol will provide an evidence map to assess if currently available literature is sufficient to define the primary exposure routes for PAEs, OPEs and NPPs, and to identify critical knowledge gaps.

3.17.P-Tu293 Temporal Patterns of Bisphenol Exposure in the Czech Population Within the Framework of Regulatory Measures

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Background: Bisphenol A (BPA), widely used in plastics and epoxy resins, has raised concern due to its ability to leach and act as an endocrine disruptor. In response, regulatory measures in Europe have increasingly restricted its use. However, since the 2010s manufacturers have started replacing BPA with analogs such as bisphenol S (BPS) and bisphenol F (BPF), which show similar endocrine activity.

This study examined temporal trends in bisphenol exposure in the Czech population and assessed whether regulatory actions targeting BPA have resulted in reduced exposure, alongside increasing exposure to its substitutes.

Methods: Urinary BPA, BPS and BPF were analyzed in two Czech cohorts sampled in 2011–2012 (DEMOCOPHES-CZ: 116 mothers, 118 children) and 2019–2020 (CELSPAC: 195 school children, 299 teenagers, 305 young adults). Statistical comparisons were made between periods and across subgroups. Population reference values were derived, and measured concentrations were compared with available health-based guidance values to evaluate potential health risks.

Results: BPA concentrations significantly decreased over time (?29% in adults; ?48% in children), while BPS concentrations increased (+121% in adults; +90% in children). Children consistently showed higher BPA levels than adults. A moderate correlation between mothers and children ($\rho = 0.34$) suggests shared household exposure. Higher levels of BPA were found in smokers in the CELSPAC adults, and children had higher levels of BPA in the warm season of the year. Additionally, females had higher BPS levels compared to males. Reference values were calculated for the Czech population. All BPA values exceeded the BPA health-based guideline value (GV) of 0.0115 µg/L and 1%-8% of BPS values exceeded the GV of 1.0 µg/L depending on cohort and age.

Conclusions: The decline in BPA and rise in BPS over time may reflect a response to BPA regulation. However, it also demonstrates the need for policy frameworks that prevent regrettable substitutions. Higher bisphenol levels in children indicate greater exposure in this group and highlight the importance of continued monitoring. Hence, human biomonitoring is essential for evaluating regulatory performance, protecting vulnerable groups such as children, and guiding future chemical policy and risk management.

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3.17.P-Tu294 Beyond Small Molecules: Adsorption Behavior of High-Molecular-Weight Polymers in Soil **Sebastian Schmiedt, Lena Mayer and Paul Bendig, Eurofins Agroscience Services, Germany**

Understanding the adsorption and desorption behavior of chemicals in soil is critical for assessing their environmental fate, including potential accumulation in soil, sediment, or sludge. OECD Guideline 106 provides a standardized approach to determine adsorption coefficients, which are essential for predicting mobility, persistence, and bioavailability of substances. These data form the basis for regulatory frameworks such as REACH to evaluate ecological risk and ensure safe chemical use.

In this study, an adapted method based on OECD Guideline 106 was applied to investigate the adsorption behavior of high-molecular-weight polymers in soils. Polyethylene glycol (PEG 1500) served as a model compound and was tested across twelve well-characterized reference soils. The adapted approach demonstrated reproducibility, enabling reliable determination of adsorption coefficients.

Results indicate that adsorption strength increases with polymer chain length and is influenced by soil properties such as texture, organic carbon content, and pH. However, due to the complexity of these factors, no clear predictive relationships could be established, indicating that polymer adsorption cannot yet be reliably predicted and must be experimentally assessed through further systematic testing.

3.17.P-Tu295 Applicability of OECD Test Guideline 120 to Various Polymers: A Comparative Evaluation and Practical Guidance

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Polymers are widely used in consumer and industrial products including textiles, paints, detergents, coatings, adhesives, and medical devices. The EU market comprises tens to hundreds of thousands of distinct polymer types. Under the EU's Chemicals Strategy for Sustainability, the Commission has proposed revising REACH to extend information and registration requirements to polymers, including standardized reporting of physicochemical properties and solubility behavior.

Existing REACH information frameworks are typically based on OECD Test Guidelines (TG). Polymers exhibit distributions of chain lengths, varied end groups, and diverse architectures. These inherent features limit the technical applicability of many current TGs and increase the risk of producing inconsistent or non-reproducible data. Polymer-specific characteristics must be explicitly addressed when applying analytical methods.

Although the OECD TG 120 on solution/extraction behavior in water focusses on polymers, it covers only certain polymer types and shows critical gaps e.g. regarding sample preparation techniques for non-solid polymers. Further standardization of the methodologies is necessary to produce consistent and comparable data sets for characterization and hazard assessments.

This comparative round robin test evaluates the applicability of the OECD TG 120 (Solution/Extraction Behaviour of Polymers in Water) to six representative polymers and polymer products, including highly viscous, liquid, and waxy materials.

Sample preparation strategies, solubility/extractability workflows, and analytical feasibility were assessed.

Technical limitations were identified and adaptations proposed to ensure robust data generation for regulatory purposes.

3.17.P-Tu296 From Calibration to Compliance - Addressing Analytical Gaps in Size Exclusion Chromatography of Polymers

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Size Exclusion Chromatography (SEC) is a cornerstone analytical technique for determining the molar mass distribution and oligomer content of polymers, which is increasingly critical as regulatory frameworks such as REACH consider extending registration requirements to polymers. Despite its widespread use, SEC faces technical limitations, which need to be considered in the context of polymer safety assessment and regulatory compliance.

This contribution, developed by the ECETOC Polymer Analytics Task Force, provides a comprehensive overview of the current state of SEC methodology for polymer analysis. We discuss the challenges associated with accurate molar mass determination, including large error margins—especially in the low molecular weight range—stemming from instrument calibration, detector limitations, and sample preparation. The recent round robin test highlighted substantial inter-laboratory variability not only for the number-average molar mass (Mn) but also the oligomer contents. These findings underscore the urgent need for harmonized protocols.

Solubility and partial solubility of polymers present further analytical challenges, as incomplete dissolution or

the presence of insoluble fractions can bias SEC results. The interference of non-polymer components, such as additives and residual monomers, complicates the quantification of low molecular weight species (<500 g/mol and <1000 g/mol), which are key parameters for regulatory thresholds. Calibration standards, experimental conditions, and detector response factors influence the accuracy and comparability of SEC data when non-polymer components are present in the sample. Which in many cases cannot be avoided for industrial samples. Based on these insights, we recommend a set of best practices for SEC analysis, including solubility assessment, careful selection of calibration standards, and subtraction/correction for side components. The harmonization of SEC methodologies and the development of standardized guidelines are essential to ensure reliable polymer characterization for regulatory and safety assessments and consequently for a safe and sustainable use of polymers in diverse applications.

3.17.P-Tu297 A Sandboxing Experiment: What if there were no Guidelines to Assess the Solubility of Polymers?

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The solubility in water is a necessary requirement for both the determination of other phys-chem parameters such as surface activity or logKow and a meaningful assessment of environmental fate. Therefore, the solubility of polymers in water has been identified as the most pressing topic the Workstream “Phys-Chem Properties and Methods” of the ECETOC Polymer Analytics Task Force has to take care of.

Since the existing guidelines are known to be not fit-for-purpose, I would like to take the opposite approach: What if there were no guidelines, but only polymer know-how and experience in the field of colloid science to study and evaluate the water solubility of polymers? This will be discussed for four different representative classes of polymers: very hydrophilic, completely water-soluble polymers, hydrophobic polymers known to form microplastic particles, polymer dispersions, and amphiphilic polymers having surfactant properties. It will be shown that one crucial requirement for the assessment of solubility of polymers in water is know-how about polymer and colloid science. Additionally, information about the composition of the polymer must be utilized to choose a suitable method – there is just no “one-method-fits-all” approach for polymers possible. This is not too surprising taking the extremely broad range of different polymer structures and properties possible into account.

3.17.P-Tu298 Uncertainty in SEC: Round Robin Test with Real-Life Polymer Samples

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Size Exclusion Chromatography (SEC) remains the most widely applied technique for determining molar mass averages and molar mass distributions of polymers. By separating molecules according to their hydrodynamic volume using a porous stationary phase, SEC provides a chromatogram that, when calibrated with standards of known molar mass, yields a relative molar mass distribution curve. From this curve, key parameters such as number-average molar mass (M_n), weight-average molar mass (M_w), and oligomer content—defined as fractions below 1000 g/mol and 500 g/mol—can be derived. These low-mass fractions are of particular regulatory interest because they represent mobile species with potentially higher bioavailability and toxicity. However, SEC is not without limitations. While error margins of 10–20% for molar mass determination across laboratories and instruments are well documented, variability in oligomer quantification may be even greater. Influencing factors include solvent choice, column configuration, dissolution behaviour, and baseline integration settings. Current DIN and ISO standards, developed from earlier interlaboratory studies, do not adequately address these challenges, especially under conditions relevant to real-life polymer samples.

To quantify these uncertainties, a comprehensive round robin test was conducted between June 2024 and February 2025 with 35 laboratories worldwide. Unlike previous studies focused on narrowly dispersed standards, this initiative included complex real-world samples such as copolymers, blends, and dispersions with gel content as a material exhibiting partial solubility. Analyses were performed in three solvent systems (THF, DMAc/DMF, and water) to reflect diverse application scenarios. The results reveal significant interlaboratory variation, particularly in the low molar mass region, with deviations exceeding 50% for M_n and even higher for oligomer content in challenging matrices. These findings underscore the urgent need for harmonized protocols, improved calibration strategies, and clear guidance on solubility and sample preparation.

This contribution presents results of the round robin study. Example cases will be discussed and interpreted to allow recommendations for method harmonization. Reducing variability in SEC polymer characterization and increasing the comparability of results are critical steps toward reliable data for regulatory compliance and polymer safety assessment.

3.17.P-Tu299 Investigating the use of Hansen Solubility Parameters for Grouping of Polymers for REACH Registration

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Although the Registration of polymers is not currently required under EU REACH, it is expected that the Commission may present legislative proposals for selecting polymers for registration in the coming years. Given the huge number of polymers on the EU market (one estimate suggests between 400,000 and >1,000,000 polymers), grouping polymers for Registration is essential to avoid placing undue burden on industry and regulators. However, no detailed proposal for how groups are to be created or validated has been put forward by the Commission.

In this work we explore the hypothesis that Hansen Solubility Parameters (HSP) may be useful in the grouping of polymers, specifically that polymers within the same structural class and in similar Hansen space will have similar (eco)toxicological properties. Developed in the 1960s, the HSP method represents solvents as points in a three-dimensional space (based on three key parameters) to predict whether one substance will dissolve in another. It has been used in the polymer industry for identifying suitable solvents for polymers. It has more recently been applied more broadly to characterise similarity between substances and how this can be used to predict their interactions with different matrices.

Hansen Solubility Parameters were calculated for various polymers including alcohol ethoxylates and methacrylates. Data visualization techniques were used to investigate potential groupings. Where available, these were compared to published classifications and/or (eco)toxicological property data.

Initial findings indicate that HSP calculations have potential to differentiate between structurally similar polymers with, for example, different molecular weights, functional group equivalent weight or oligomer content. The need for good quality (eco)toxicological data for structurally defined polymers to validate potential groupings is highlighted and the uncertain reliability of current classifications of polymers is identified as a key variable.

3.17.P-Tu300 Integrating Safe and Sustainable by Design Criteria for Regulatory Assessment of Emerging Biopolymers in Packaging Applications

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The transition toward biodegradable polymers requires robust and integrated approaches to ensure that new materials are safe, high performing and environmentally sustainable. The Be-UP project addresses this challenge by developing innovative biopolyesters, materials and packaging prototypes and evaluating them through a comprehensive Safe and Sustainable by Design (SSbD) methodology. A major barrier to the adoption of biodegradable polymers is the absence of harmonised frameworks that link material performance with environmental fate, human health considerations and circularity. Be-UP aims to overcome this gap by implementing a full SSbD assessment, integrating environmental, economic, social and safety dimensions. The SSbD Framework is applied from early development to final demonstrators, defining criteria for safety, sustainability, recyclability and risk assessment. A multi-criteria decision tool is being developed to combine information on environmental impacts, human health risks and life cycle performance, enabling dynamic comparison of materials and guiding design choices for researchers and industry. Complementary studies assess techno-economic feasibility across the value chain, including market context, mass and energy balances and economic performance supported by life cycle costing. Sustainability performance is addressed through life cycle assessment and social life cycle assessment, comparing conventional production and end-of-life scenarios with the novel Be-UP approach.

Recyclability is evaluated through mechanical and chemical routes, assessing material integrity over repeated processing cycles and the potential for depolymerisation and re-polymerisation into high-quality products. Risk assessment integrates toxicity testing of additives and final materials and ecotoxicity studies in aquatic and terrestrial environments. It also considers the potential release of micro- and nanoplastics and their role in biodegradation processes and biological interactions.

Together, these activities deliver a harmonised framework for assessing biodegradable polymers, linking fate, performance and sustainability. The results will support the safe implementation of new bioplastics and inform future best practices for polymer design and regulation.

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3.17.P-Tu301 Pitfalls of Ambiguity in the Development of Safe and Sustainable Biobased Plastics: How Can Industry and Regulators Better Navigate this Landscape?

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Plastics regulation in the European Union has expanded rapidly in response to growing concerns about pollution, human exposure, and the rise of alternative materials. Yet, definitions of plastics, polymers, and biobased materials remain fragmented and sometimes ambiguous, creating uncertainty for scientists and developers. This study examines how plastics and biobased plastics are defined and regulated within the EU, with the aim of clarifying key terms and helping researchers navigate the policy landscape more effectively. We first map all relevant EU regulations that apply to plastics across major sectors and identify where biobased and biodegradable materials are treated similarly or differently from conventional plastics. We then analyse how regulatory definitions, such as “polymer,” “plastic,” “biobased,” “natural polymer,” and “chemically modified” are applied across policies. Building on two existing classification frameworks, we develop a simplified decision tree that helps determine whether a material qualifies as a plastic under EU law and whether it falls under the microplastic restriction.

To test its usefulness, we apply the decision tree to several emerging biobased materials, including cellophane, protein-based polymers, lignin, biobased microcapsules, and rubber. These case studies reveal where regulatory interpretations diverge and where chemical modification becomes a decisive factor.

Our results show that most biobased polymers are regulated in the same way as conventional plastics unless they meet strict criteria for being natural and not chemically modified. This highlights the importance of early regulatory consideration during material design. With the decision tree we aim to propose a practical tool to support this process by making regulatory boundaries more transparent. Overall, the study contributes to clearer communication between regulators and innovators and supports the development of safer and more sustainable biobased materials within a rapidly evolving policy landscape.

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3.17.P-Tu302 Are bioplastics safer: An in vitro approach

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Nanoplastics are emerging environmental contaminants, and humans are a potential target of their effects. These particles are ubiquitous in the environment and have been detected in food items (e.g., seafood) and beverages, including drinking water, with evidence of human exposure demonstrated by their presence in urine, stool, blood, and tissues such as the lung, liver, and brain. One of the major limitations in the current research addressing the potential effects of nanoplastics is the predominance of studies using pristine polystyrene (PS) nanoplastics, which fail to account for the diversity of polymer types found in the environment. There is a clear need to assess the potential of biopolymers as a safer alternative to the classic fossil-based polymers.

This study focused on the effects of polyethylene terephthalate (PET) (fossil-based) and polyhydroxybutyrate (PHB) (bio-based) nanoplastics on HepG2 (hepatocyte-like – to assess effects on a metabolic model) and HCT116 (intestinal epithelium – representing a critical barrier against their systemic entry into the body). PET is one of the most produced polymers worldwide and the polymer most used for water bottles, whereas PHB is a biodegradable polymer (made by bacteria) marketed as environmentally friendly, although its effects have not been thoroughly studied.

Cells were incubated with PET (191 nm) and PHB (195 nm) at the concentrations ranging from 100 to 0.0064 mg/L and from 50 to 0.0032 mg/L, respectively (5-fold dilution factor). Effects on cell viability (MTT and LDH assays) were assessed at 24, 48 and 72h. Overall, LDH levels in the media were not significantly different in any of the conditions, showing no significant cell death. The MTT results indicated a gradual decline of cell metabolism at 24 and 48h but recovered at 72h. In HepG2, PHB nanoplastics elicited a higher impact than PET nanoplastics.

Overall, these preliminary findings indicate transient stress, without cell death, and suggest that biopolymers are

not risk-free. Ongoing essays aim to clarify these effects, examining how these particles influence overall cell function.

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3.17.P-Tu303 Biopolymers: Ecotoxicological Risks of Micro and Nanoplastics Aquatic Organisms

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Intense plastic production and poor waste management are promoting the widespread accumulation of microplastics (MPs) and nanoplastics (NPs) in aquatic ecosystems. These size fractions, that disperse through the water column, sediments and biofilms, display high mobility, environmental persistence, and bioavailability, enabling uptake by diverse aquatic taxa, ranging from plankton to higher trophic levels (e.g. fish and humans). The reported effects on aquatic organisms include feeding disruption, oxidative stress, inflammation, metabolic dysregulation, and reproductive impairment. The reported effects on aquatic organisms include feeding disruption, oxidative stress, inflammation, metabolic dysregulation, and reproductive impairment. NPs, due to their small size, ability to penetrate cells, form eco-coronas, and facilitate co-transport of sorbed contaminants, are considered potentially more hazardous to biota.

Biopolymers such as polyhydroxyalkanoates (PHAs) and polylactic acid (PLA), derived from renewable, biological feedstocks, are increasingly proposed as sustainable alternatives to fossil-based polymers. Although expected to degrade in the environment, their degradation rates remain highly context dependent, and environmental fragmentation can still generate micro- and nano-sized particles. As industry moves toward nanoscale formulations, nano-biopolymers introduce new exposure pathways for freshwater organisms. The available studies on nano-biopolymers have reported size- and surface-dependent uptake, interactions with epithelial and digestive tissues, and potential oxidative or inflammatory responses. Critical knowledge gaps persist regarding environmental transformation, environmental levels, biodegradation kinetics and trophic transfer potential of nano-biopolymers in freshwater ecosystems. Furthermore, comparative ecotoxicological data on nano-biopolymers relative to synthetic NPs remain scarce. This study aims to synthesize available evidence on the ecotoxicological impacts of nano-biopolymers in freshwater organisms and evaluate if these polymers are in fact safer and more sustainable than conventional fossil-based.

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3.17.P-Tu304 Understanding Life-Stage Susceptibility to Biodegradable Nanoplastics: A Case Study in Hediste diversicolor

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Biodegradable polymers have been proposed as more alternatives to conventional plastics. However, growing evidence suggests that their fragmentation into micro- and nanoplastics (NPs) raising significant concern about potential ecological risks. Polyhydroxybutyrate (PHB), a prominent biopolymer of the polyhydroxyalkanoate family, can generate NPs whose effects on key aquatic organisms remain poorly understood. This study investigated the impacts of PHB NPs exposure on juvenile and adult polychaete *Hediste diversicolor*, a key bioturbator species in estuarine ecosystems. Organisms were exposed for 14 days to sediments spiked with PHB NPs (103.9 ± 5.07 nm) at 0, 0.5, 2, 8, 32, 128 mg PHB/kg sediment. Behavioral (feeding, burrowing, light

avoidance, bioturbation) and physiological (regenerative capacity) endpoints were assessed, after exposure and a 7 day depuration in clean sediment.

The results demonstrated that PHB NPs exposure altered behavior in both life stages, with juveniles showing higher sensitivity and reduced resilience. Feeding activity was significantly impaired in exposed polychaetes, with juveniles requiring more time to detect and capture food. Burrowing activity was impaired in juveniles (32 mg/kg) and in adults (128 mg/kg), though both stages recovered after depuration. Light avoidance responses were significantly delayed in juveniles and adults, with juveniles failing to recover at the highest concentration. Regarding bioturbation activity, sediment remobilization was significantly reduced across all juveniles' treatments, and persisted after the recovery period, compromising sediment oxygenation and nutrient cycling in estuarine habitat. Adults exhibited significant reductions at high concentrations, with partial recovery. Regarding regenerative capacity, adults exposed to PHB NPs regenerated fewer segments and showed reduced overall regeneration percentage across all treatments.

Overall, the findings highlight that PHB NPs impacted behavioral and physiological endpoints in *H. diversicolor*, with juveniles exhibiting reduced resilience and being unable to completely recover after 7 days. These findings demonstrate that even biopolymer particles can pose ecologically relevant risks at the nanoscale, potentially compromising sediment dynamics, nutrient cycling, increasing vulnerability to predators and population fitness.

3.17.P-Tu305 Synthesis of Metal-Embedded Nanoplastics as Test Materials and Quantification by spICP-MS

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Conventional approaches for characterizing nanoplastics and assessing their behavior in environmental matrices have primarily relied on commercially available plastic particles and analytical techniques such as dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), μ -FTIR, and Raman spectroscopy. However, these methods face significant limitations in complex environmental samples, particularly in distinguishing nanoplastics from background particles or carbon-based materials and in accurately quantifying particle number concentration (PNC). Recently, a new analytical strategy has emerged in which nanoplastics are labeled with metals and quantified using single-particle inductively coupled plasma mass spectrometry (spICP-MS), allowing the determination of particle size distribution (PSD) and PNC based on individual metal-derived signal events. This study aims to synthesize metal-embedded nanoplastics (Me-NPs) and establish a quantitative analytical method utilizing their metal signatures. Me-NPs were produced by incorporating metals (e.g., tantalum (Ta), gold (Au)) into commonly used polymer resins, including polystyrene (PS), polyvinyl chloride (PVC), and polypropylene (PP). To fabricate these materials, both top-down approaches (e.g., cryogenic milling, chemical fragmentation) and bottom-up approaches (e.g., oil-in-water (O/W) emulsion solvent evaporation, antisolvent nanoprecipitation) were employed. Using the element specificity of spICP-MS, we developed an analytical framework capable of determining the PSD and PNC of the synthesized Me-NPs. These Me-NPs are expected to serve as test materials that can be utilized in future investigations aimed at examining the environmental behavior of nanoplastics—such as weathering, aggregation, dispersion, and mobility—under various environmental conditions.

3.17.P-Tu306 Nanoplastics in agriculture: ingestion and impacts on arthropods in food webs

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Nanoplastics (NPs) are of growing concern due to their uptake by organisms, ability to cross biological barriers, and potentially greater toxicity compared to microplastics. Although awareness of their ecological risks is increasing, the ingestion and impacts of NPs within terrestrial ecosystems remain poorly understood, especially compared to aquatic systems. Assessing NP exposure in soil-dwelling organisms is particularly challenging because of methodological limitations in quantifying NPs at low concentrations and the complexity of exposure routes. Building on our previous development of a palladium (Pd)-doped NP exposure system for agricultural food webs, this study expands the approach to additional arthropod species. We established NP exposure assays for four representative terrestrial arthropods: an omnivorous rove beetle (*Dalotia coriaria*), a carnivorous devil's coach-horse rove beetle (*Ocypus olens*), a herbivorous mealworm beetle (*Tenebrio molitor*), and a detritivorous springtail (*Folsomia candida*). Adult rove beetles, mealworms, and springtails were exposed to Pd-doped NPs via spiked dry yeast feed for short-term (up to 1 week) and/or long-term (up to 6 weeks) durations, while rove

beetle larvae were exposed throughout their developmental period. Larvae of the devil's coach-horse beetle were additionally fed NP-exposed mealworms for up to 1 week. NPs in all species were quantified using inductively coupled plasma–mass spectrometry (ICP–MS), with Pd serving as a tracer. Using these measurements, we compared NP burdens across species to evaluate bioaccumulation and assess toxicity via biological endpoints including growth, reproduction, and mortality. We also compared NP burdens across developmental stages to examine changes associated with metamorphosis, and between predator–prey pairs to evaluate the potential for trophic transfer and to estimate biomagnification factors. This study provides new insights into the fate and effects of NPs in arthropods within agricultural food webs, demonstrating the utility of Pd-doped NPs and supporting the development of ecological risk assessment frameworks in terrestrial environments.

3.17.P-Tu307 Assessment of Nanoplastic Pollution in Aquatic Systems from Wastewater Sources in Eastern Germany

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The unchecked littering and mismanagement of plastics, coupled with their rising production and usage, have escalated them into one of the most pressing environmental pollutants. Among them, microplastics (<5 mm) and nanoplastics (<1 µm) have emerged as critical contaminants, with NPs posing the greatest risks due to their ability to penetrate and contaminate water sources. While MPs are globally found to contaminate every freshwater ecosystem, it is reasonable to expect NPs to be similarly widespread as a result of MPs degradation with impacts still unknown. Wastewater systems such as wastewater/sewage treatment plants (WWTPs/STPs) are increasingly recognized as major pathways for nanoplastics, facilitating their re-entrance into river systems. Importantly, such land-based discharges of nanoplastics into rivers ultimately contribute to the growing plastic pollution load in oceans, linking inland sources directly to marine contamination. Globally, numerous studies have examined the abundance, pathways, and removal efficiencies of microplastics in WWTPs/STPs; however, systematic assessments of nanoplastics remain scarce. In Europe, despite increasing awareness of microplastic pollution in rivers like the Rhine, Elbe, and Danube, no study to date has focused on the occurrence, size-wise distribution, and fate of nanoplastics in wastewater systems and river water. Henceforth, the present study aims to address this gap by investigating nanoplastic pollution in raw and treated wastewater from WWTPs in Leipzig, which ultimately discharge into the Elbe River, using highly sensitive techniques such as thermal desorption–proton transfer reaction–mass spectrometry (TD-PTR-MS). By establishing the first comprehensive dataset of nanoplastic concentrations in these environmental matrices, the study will generate new insights into their fate and transport, inform technological upgrades in treatment systems, and guide policymakers in developing evidence-based regulations and guidelines to mitigate nanoplastics pollution.

3.17.P-Tu308 Ultra-sensitive quantification of mixed nanoplastic particles from environmental water samples through magnetic enrichment using custom-designed bifunctional peptides

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Plastic materials such as PE, PP, PS, and PET are extensively used and increasingly discarded into the environment. The degradation of synthetic polymers in a circular polymer economy inevitably leads to the formation and widespread release of micro- and nanoplastics (MP/NPs), making the management of plastic contamination in aquatic environments crucial. NPs under 1 µm pose specific risks due to high surface area and colloidal stability, especially with those under 20 nm capable of crossing biological barriers. Detection and quantification of these small particles, especially those smaller than 5 nm, remain major analytical challenges acknowledged by the EU Commission, hindering the establishment of effective regulatory policies. Conventional techniques such as FTIR, Raman, and dynamic light scattering are limited in sensitivity or polymer specificity. On the other hand, membrane-based separations often fail to capture the smallest NPs, underestimating total loads. To overcome these limitations, we developed a magnetic enrichment platform by functionalizing Fe₃O₄ superparamagnetic nanoparticles (SPION) with custom-designed bifunctional peptides capable of binding a broad range of polymer types, followed by analysis via Py-GC/MS. We assessed enrichment efficiency, adsorption kinetics, adsorption isotherms, and salt effects using model NP suspensions, and further evaluated real-world applicability by spiking mixed NPs into 1 L environmental water samples, followed by analysis of seven types of environmental waters (river, lake, seawater, wastewater). The system achieved over 96 % recovery for PS, PP, PE, and PET NPs, with a maximum adsorption capacity of 3.95 ± 0.14 g NPs · g⁻¹ adsorbent for PS-COOH500 nm. Adsorption was enhanced by salt ions, where hydrophobic interactions served as the primary driving force for NPs binding, demonstrating the platform's versatility across diverse aqueous environments. The method successfully enriched, detected, and quantified mixed NPs - including PP, PE, PS, PET, PMMA, PC, nylon 6, and nylon 66 - from complex water matrices, with a detection limit down to 0.061 µg for PS, demonstrating broad applicability for environmental monitoring. This work presents a sensitive, versatile, and efficient approach for isolating and quantifying NPs in aquatic environments,

thereby supporting improved assessment of nanoscale plastic pollution and informing regulatory and mitigation strategies.

3.17.P-Tu309 Physiological and Compositional Effects of Poly(3-Hydroxybutyrate) and Polymethyl Methacrylate (PMMA) Nanoplastics in Invertebrates

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Micro- and nanoplastics (MNPs) are ubiquitous in marine systems, arising from diverse sources, such as the fragmentation of larger plastic debris, direct emissions from industrial processes and consumer products, and biological activity. However, the toxicokinetics and physiological impacts of both conventional and biodegradable polymers remain poorly understood. In this study, we evaluated the toxicokinetics and biological responses of the mussel "*Mytilus galloprovincialis*" to two environmentally relevant nanoplastics: polyhydroxybutyrate (PHB, 200 nm) and polymethylmethacrylate (PMMA, 250 nm) following a preliminary investigation of their effects on the microalga "*Tetraselmis suecica*".

No acute toxicity to the microalgae was observed across a wide concentration range of both MNPs (4.69–150 mg/L²¹). However, All nanoplastic treatments resulted in stoichiometric disruptions after 24h, with co-exposure of both nanoplastics showing a 30% reduction in nitrogen content, elevated C:N ratios, and $\delta^{13}\text{C}$ enrichment consistent with photosynthetic stress. In mussels, gene expression responses were tissue- and polymer-specific: CAT was upregulated in the digestive gland after PHB and remained elevated under co-exposure, while GST- π in gills responded only to the mixture. PHB and PMMA induced distinct biological effects with PMMA particularly triggering oxidative pathways. Py-GC/QTOF-MS detected both polymers in faeces but not in tissues, indicating rapid elimination and limited short-term bioaccumulation.

Our findings underscore the need for integrated stoichiometric, transcriptomic, and chemical approaches to assess ecological risks of emerging nanoplastics under realistic exposure scenarios.

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3.17.P-Tu310 Flow Cytometry as a Non-Destructive Tool for Detecting Micro- and Nanoplastics in Complex Biological Samples

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Micro- and nanoplastics (MNPs) have been detected in human tissues and biological fluids, including blood, yet little is known about their distribution in the body or how they interact with immune cells. Current analytical methods rely on harsh digestion or high temperature procedures that destroy cellular structures, making it impossible to study particle-cell associations in intact biological samples. To address this challenge, our objective was to develop a sensitive, non-destructive method capable of detecting MNPs in biological matrices while preserving immune cell architecture. We established a flow cytometry assay using optimized Nile Red fluorescence to detect MNPs in blood while maintaining intact immune cells. Validation was performed with polystyrene particles of 0.5, 1, and 3 μm , and ongoing work extends the method to additional polymers, including polyethylene and poly(methyl methacrylate). Analytical performance metrics - limit of detection, limit of quantification, sensitivity, and specificity - are being determined to support comparison with pyrolysis-gas chromatography-mass spectrometry. Although initially developed for blood, the assay is being adapted to other clinically relevant matrices, including atherosclerotic plaques, to enable the study of particle-cell associations across different tissue environments. Its compatibility with multiparameter immunophenotyping will support future assessments of whether MNPs preferentially associate with specific immune cell subsets or inflammatory profiles. The assay reliably identifies polystyrene particles with clear separation from background signals, and validation across multiple particle sizes demonstrates robustness. Establishing sensitivity and specificity supports applicability for trace-level biomonitoring across diverse sample types. This non-destructive flow

cytometry approach provides a practical and accessible tool for detecting MNPs in complex biological samples while preserving immune cell structure. Because flow cytometers are routinely available in clinical laboratories, the method can be readily integrated into existing workflows for large-scale human monitoring. Extending this assay to vascular tissues such as atherosclerotic plaques will support studies exploring potential links between particle presence and inflammatory processes, advancing research on human exposure and the health impacts of plastic pollution.

3.17.P-Tu311 Museum Foraminifera Archive Historical Marine Plastic Pollution

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Historical reconstruction of marine plastic pollution is essential for understanding long-term ecological impacts, yet suitable natural archives remain scarce. Agglutinated foraminifera, benthic protists that incorporate surrounding sediment into their shells (tests), offer untapped potential to record the emergence and rise of micro(nano)plastics in marine systems. The goal of this study was to determine whether foraminiferal tests preserve quantifiable plastic-derived polymers and to evaluate their suitability as century-scale plastic pollution indicators. Using pyrolysis–gas chromatography–mass spectrometry with matrix-matched calibration, we analyzed historical agglutinated foraminifera samples (*Rhabdammina discreta*, *Astrorhiza furcata*, *Conglophragmium* sp., and *textulariid*) collected from Departure Bay, the Norwegian Sea, and the Puerto Rico Trench between 1911 and 1952. Procedural blanks, solvent-free preparation, and polymer-spiked controls were used to assess contamination risk, instrument sensitivity, and method performance. Quantified plastic-derived polymers exhibit a temporal increase consistent with global trends in plastic production. By 1952, elevated concentrations were observed, including PE at up to 78.7 µg/g, PS at up to 0.9 µg/g, and PET at up to 45.0 µg/g. Our findings establish foraminifera as potential indicators of past pollution, providing a rapid, novel method for tracking long-term marine pollution using historical museum specimens and enhancing global environmental monitoring.

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3.17.P-Tu312 Temporal variability and risk assessment of microplastics in surface water from a coastal lagoon, southern Gulf of Mexico

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This study assesses the abundance, characteristics, and ecological risk of microplastics (MPs) in the Sontecomapan Lagoon System (SLS), a RAMSAR site in the southern Gulf of Mexico. MPs, defined as particles <5 mm, are emerging pollutants that can carry toxic substances and cause sublethal effects in aquatic organisms. In tropical regions, their concentration varies seasonally, increasing during the rainy season due to greater continental runoff and atmospheric precipitation.

The lagoon was sampled on three occasions: during the rainy season (June 2018, October 2019) and the dry season (March 2023) at ten stations within the lagoon and the rivers that flow into. MPs were obtained by sieving, organic matter digestion, and density separation, and were characterized by microscopy and attenuated reflectance Fourier transform infrared spectroscopy (µFTIR-ATR). Ecological risk was assessed using the PLI, PHI, and PERI indices. Concentrations were higher in June (7.5 items/L) than in October (1.7 items/L) and March (1.5 items/L), confirming an increase during the rainy season. Inland tributaries registered 5.2 items/L, highlighting their role as entry points for plastic pollution into the lagoon. Particles smaller than 1000 µm were dominant (58–83%), increasing their bioavailability to zooplankton. Fibers were the most frequent form (56–95%), mainly associated with textiles and fishing activities, and the color blue predominated in all sampling campaigns. While the PLI indicated a low pollutant load, the PHI and PERI indices revealed risks ranging from

low to extremely high, mainly due to the presence of hazardous polymers such as acrylic. High risk levels imply a high probability of adverse effects on pelagic organisms, altering population structure and dynamics, and ultimately disrupting the functioning of the food web. In conclusion, despite their low abundance, microplastics (MPs) pose a significant risk due to their chemical composition and spatial distribution. Priority areas for monitoring and mitigation are identified, and it is emphasized that the risk posed by microplastics should be assessed not only by quantity but also by the material's hazardous nature.

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3.17.P-Tu313 Microplastic-Biofilm as Hotspots for Antibiotic Resistance in Hospital Wastewater: An Emerging Environmental Health Concern

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The intersection of plastic pollution and antimicrobial resistance (AMR) in hospital wastewater (HWW) poses a growing threat to public health. These effluents are known to contain a high load of both antimicrobials and pathogens. Additionally, microplastics (MP) have been reported to be stable substrates allowing the attachment of microorganisms, referred to as the plastisphere. By offering both a substrate for increased horizontal gene transfer and a protective niche for biofilm formation, the presence of MP in the HWW could accelerate the spread of AMR. Despite the high risks associated with untreated clinical discharge, the specific role of MPs as vectors for resistance in hospital wastewater remains poorly understood. This research investigates the chemical nature of MPs in these effluents and their capacity to support resistant microbial communities.

Using untreated wastewater samples from a major hospital, we collected parallel samples to conduct chemical and biological analyses independently. In this study we characterized the MP pollution profile via 10% KOH digestion and FTIR spectroscopy, and determining microbial DNA concentrations following 16S/18S rRNA PCR amplification. Microorganisms were isolated from MPs and their antibiotic susceptibility profile was determined. FTIR analysis and cultured- based assays suggest detailed insights into the size distribution of these vectors, the extent of eukaryotic vs. prokaryotic colonization, and the comparative resistance profiles of attached vs. free-living bacteria will be further analyzed. Overall, this research bridges environmental chemistry and microbial ecology to reveal how plastic pollution intersects with antimicrobial resistance in clinical effluents, underscoring a pressing need for One Health-oriented wastewater mitigation strategies.

3.17.P-Tu314 Screening Level Environmental Risk Assessment of Microplastics in Dutch Waters

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Microplastics by now seem to be ubiquitously present in our environment. Tackling plastics pollution requires effective mitigation measures. One common way of prioritising such measures in chemical regulatory frameworks is to base them on the outcomes of environmental risk assessments. This is not common practice yet for microplastics, although the first risk assessments are already available in scientific literature. For this reason we performed a screening level study into the environmental risk of microplastics in Dutch waterbodies. This was done to learn about the best practices to perform a regulatory relevant environmental risk assessment (RIVM report 2025-0095) and to get a better understanding of the extent of microplastic pollution in the Netherlands. To this end, we used existing environmental measurements, both spectroscopic and pyrolysis-based. These were recalibrated to the 1 to 5000 µm size range and compared to literature reported HC5 levels that were derived for the same range of particle sizes based on volume or surface area as the environmentally relevant metric. Several challenges are identified, e.g. in recalibration of pyrolysis based data for comparison to HC5 levels. This work illustrates the need for a better understanding of microplastics size distributions in the environment and the need for more refined measurements spanning a larger size range of microplastics.

Disclaimer/Disclosure:

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3.17.P-Tu315 Toxicological Interactions Between Polystyrene Microplastics and Coastal Pollutants in the Marine Bivalve *Crassostrea gigas*

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This study investigated the toxicological interactions between microplastics and representative coastal environmental pollutants, specifically cadmium (Cd) and phenanthrene (PhA), to better understand the potential environmental risks posed by microplastics through combined toxicity.

Exposure experiments using the marine bivalve *Crassostrea gigas* were conducted to assess fertilization rate, D-shape embryogenesis incidence, and survival rate. Microplastics alone did not exhibit any significant toxic effects on these three biological endpoints across all tested concentrations.

However, both Cd and PhA showed clear concentration-dependent toxic effects, following a sigmoidal dose-response curve. When exposed to Cd alone, the EC50 values for fertilization rate, D-shape embryogenesis, and survival rate were $12.768 \pm 0.256 \text{ mg}\cdot\text{L}^{-1}$, $3.592 \pm 0.136 \text{ mg}\cdot\text{L}^{-1}$, and $> 40 \text{ mg}\cdot\text{L}^{-1}$, respectively. In contrast, co-exposure to Cd and microplastics resulted in EC50 values of $14.429 \pm 0.206 \text{ mg}\cdot\text{L}^{-1}$, $3.899 \pm 0.083 \text{ mg}\cdot\text{L}^{-1}$, and $27.500 \pm 1.804 \text{ mg}\cdot\text{L}^{-1}$ for the same endpoints, indicating a slight reduction in Cd toxicity in early life stages but a significant increase in toxicity at the adult stage. This suggests that microplastics may facilitate Cd bioavailability by acting as vectors or carriers.

Similarly, exposure to PhA alone resulted in EC50 values of $0.038 \pm 0.015 \text{ mg}\cdot\text{L}^{-1}$, $0.006 \pm 0.001 \text{ mg}\cdot\text{L}^{-1}$, and $> 0.2 \text{ mg}\cdot\text{L}^{-1}$ for fertilization, embryogenesis, and survival, respectively. When PhA and microplastics were co-exposed, the EC50 values changed to $0.076 \pm 0.033 \text{ mg}\cdot\text{L}^{-1}$, $0.022 \pm 0.001 \text{ mg}\cdot\text{L}^{-1}$, and $> 0.2 \text{ mg}\cdot\text{L}^{-1}$, respectively. Interestingly, in contrast to Cd, the presence of microplastics appeared to decrease PhA toxicity in both larval and adult stages.

These results indicate that microplastics may modulate the toxicity of co-existing pollutants depending on their chemical properties. Hydrophobic contaminants such as PhA may show reduced toxicity through sorption to microplastics, whereas metals like Cd may exhibit increased toxicity due to facilitated transport and bioaccumulation. Thus, microplastics could act as both passive carriers and active participants in pollutant dynamics, ultimately influencing the ecological risk to marine organisms such as *C. gigas*.

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3.17.P-Tu316 Analyzing the Effect of UV-C Exposure on the Microplastics Particles from Wind Turbine Blades

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Wind turbines are among the leading renewable energy sources in Europe and have expanded significantly over the past few decades. One of the main challenges associated with wind energy is the continuous operation of turbines under varying environmental conditions, including high-speed rain, strong winds, and UV radiation. Repeated exposure to these conditions can affect the coating surface of the blades. This coating has the potential to degrade into microplastics, which can then disperse into the air and eventually deposit in the marine environment. However, there is still limited research analyzing the behavior of these microplastics in the environment and their effects at the microscopic level. In this study, we aimed to analyze the fate of microplastics from wind turbine blades under accelerated aging conditions using UV-C exposure. We used ground material from the maintenance of wind turbine polyurethane coatings, representing microplastic particles released from the blades. These polyurethane particles were then placed in artificial seawater to evaluate the effects of UV exposure. The experiments were conducted for 7, 14, and 28 days of UV-C exposure, corresponding to approximately 25, 50, and 101 days of natural sunlight exposure. Samples were then analyzed with micro-FTIR-imaging spectroscopy. Preliminary results revealed that after 28 days of UV-C exposure, the particles exhibited visible yellowing and chemical changes consistent with the breakdown of the polyurethane structure. This degradation was indicated by increased absorbance at 1714 cm^{-1} and 3343 cm^{-1} , along with decreased peaks at 2924 cm^{-1} and 2825 cm^{-1} . These findings indicate that UV exposure accelerates the deterioration of polyurethane coatings, leading to the formation of more oxidized and potentially more fragile

microplastic fragments. Understanding these degradation mechanisms is therefore essential for evaluating the full life-cycle impacts of wind turbine materials and for guiding the development of more durable and environmentally sustainable coatings for next-generation renewable energy infrastructure.

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3.17.P-Tu317 Monitoring the Release of Plastic Chemical Additives from Conventional and Biodegradable Microplastics using Outdoor Mesocosms

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The extended use of plastics in agriculture has led to the release of large concentrations of microplastics (MP) into freshwater ecosystems through surface runoff, throughflow, or windthrow. However, the release of plastic chemical additives (PCAs) from conventional and biodegradable MPs in aquatic ecosystems remains poorly understood. PCAs comprise a variety of organic compounds with different physical-chemical properties, functional groups, and molecular sizes. This, combined with the vast number of chemical compounds classified as PCAs, underscores the need for robust methodologies to study exposure to these contaminants and to assess the associated risks to environmental and human health. The objective of this work was to monitor the release of PCAs from conventional (polyethylene) and biodegradable (polybutylene adipate terephthalate) MPs generated from mulch films used in agriculture using a realistic setup. For this purpose, both MPs were separately added to 1m³ fiberglass outdoor mesocosms at environmentally relevant concentrations for three months. To identify the presence of PCAs in both polymer types, MPs were first analysed by pyrolysis coupled to gas chromatography-mass spectrometry (Py-GC-MS) without any sample treatment. The pyrolysis temperature was set at 450°C, which allow to adequately identify PCAs without interferences coming from the pyrolysis fragments of the plastic polymers. As a result, the number of PCAs detected in MPs was as follows: 10 in bioplastic mulch, 19 in polyethylene mulch, and only one detected in both two materials. Fatty acids were the predominant PCAs present in both materials. Phosphated and nitrogenated PCAs were only identified in polyethylene mulch. A total of 15 PCAs detected in MPs were chosen to develop a quantitative method in PCAs release to the water by extraction with stir bar sorptive and subsequent analysis by GC-MS. This method will be applied to analyse PCAs in the water samples collected over time.

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3.17.P-Tu318 Influence of Combustion Scale on Size-Resolved PAH Emissions and Cytotoxicity from Polystyrene Fires

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Plastic waste combustion is a major source of hazardous emissions, yet its health impacts remain insufficiently understood. Polystyrene, widely used in packaging and consumer products, releases substantial soot and polycyclic aromatic hydrocarbons (PAHs) during burning. These particles can penetrate deep into the lungs and induce oxidative stress, inflammation, and respiratory injury. However, the toxicological properties of PAH mixtures generated from polystyrene under realistic fire conditions have not been systematically characterized. This study evaluated how combustion scale affects PAH emissions and cytotoxicity. Polystyrene was burned in an ISO 9705 fire test room using 20-cm and 60-cm pans. Aerosols were collected with an eight-stage cascade impactor and separated into coarse and fine fractions. Extracts were analyzed for total PAHs, compositional

profiles, benzo[a]pyrene equivalents, and excess lifetime cancer risk. Human bronchial epithelial BEAS-2B cells were exposed to the extracts, and 24-hour cytotoxicity was measured using the MTT assay. PAH concentration differences were assessed with the Mann–Whitney U test, and cell viability with the t-test, both conducted in R 4.5.0.

Fine particles were the dominant carriers of PAHs at both combustion scales. In the 20-cm fire, fine-fraction PAH levels were four to five times higher than those in the coarse fraction, while in the 60-cm fire they were roughly twice as high. Scaling combustion from 20 to 60 cm substantially increased PAH concentrations across all size fractions. Fine particles were enriched with four- and five-ring PAHs, whereas coarse particles contained lower proportions of these high-molecular-weight compounds. Corresponding benzo[a]pyrene equivalents indicated lifetime cancer risks of ~10⁻⁷ for the 20-cm fire and ~10⁻⁸ for the 60-cm fire. BEAS-2B cells exposed to 0.5% extracts showed 10–20% viability loss for 20-cm combustion and 40–50% for 60-cm combustion. Future work will incorporate PAH diagnostic ratio analysis, evaluation of combustion parameters and size-resolved mass distributions, and quantification of inflammatory cytokines—including IL-6 and aryl hydrocarbon receptor-associated proteins (AHR, CYP1A1, CYP1A2, CYP1B1)—via western blot to develop a more comprehensive toxicological profile.

3.17.P-Tu319 Combustion-Generated Phthalates and PAHs from Polypropylene: Size-Dependent Chemical Profiles and Lung Cell Toxicity

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Open burning of plastic waste emits hazardous organic pollutants, including polycyclic aromatic hydrocarbons (PAHs) and phthalate esters (PAEs). Although research has largely focused on PAH toxicity, emerging evidence indicates that PAEs can persist under incomplete combustion and contribute to oxidative and inflammatory effects. This study characterized pollutants produced from polypropylene combustion and assessed their cytotoxic and pro-inflammatory potential in BEAS-2B lung epithelial cells. Combustion tests were conducted at the Fire Laboratory of the National Kaohsiung University of Science and Technology following ISO 9705 procedures. Emissions were sampled at 20 cm and 60 cm above the flame, extracted with organic solvents, and analyzed using gas chromatography–mass spectrometry. BEAS-2B cells were exposed to the extracts for 24 hours, and cytotoxicity was measured using the MTT assay. Statistical analyses were performed in R version 4.5.0.

Chemical profiles varied by sampling height and particle size. While total particle mass did not differ significantly, fine particles at 60 cm contained higher PAH concentrations, especially 4–5 ring species such as fluoranthene and pyrene. Phthalate esters showed similar enrichment, with di(2-ethylhexyl) phthalate concentrated in fine particles at 60 cm, suggesting preferential condensation of high-molecular-weight compounds. MTT results demonstrated dose-dependent reductions in BEAS-2B viability, with the strongest effects observed in fine-particle extracts collected at 60 cm. However, non-cancer risk assessment showed hazard quotients for diethyl phthalate, dibutyl phthalate, and di(2-ethylhexyl) phthalate were all below 1, indicating low expected risk under environmental exposure conditions.

The next phase of this work will examine inflammatory responses induced by phthalate esters in lung epithelial cells. Interleukin-6 secretion will be quantified using ELISA, and Western blotting will evaluate CYP1A1, CYP1A2, CYP1B1, and aryl hydrocarbon receptor expression. These analyses will clarify inflammatory pathways and determine whether AhR-mediated signaling contributes to IL-6 regulation following phthalate exposure.

3.17.P-Tu320 The Trojan Horse: Exploring Release of Chemicals from Plastics into the Ocean

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The extensive global production and use of plastics and chemicals have led to increasing triple planetary crisis. Large quantities of chemicals in plastics are transported into natural environments through plastic leakage and eventually into the oceans, posing long-term risks to marine ecosystems. However, their environmental burden remains unquantified globally. This study developed a mass transfer model to quantify the release of chemicals from plastics into the oceans based on Fick's diffusion law, incorporating the impact of plastic aging. A assessment framework was established through coupling the mass transfer model and dynamic material flow

analysis (DMFA). It was then applied to quantify the global release of nine chemicals of concern (CoCs) from plastics into the oceans, including Penta-BDEs, Octa-BDEs, Deca-BDEs, HBCD, DEHP, DINP, SCCPs, MCCPs and TCPP.

The results show that, between 2000 and 2020, the global mass of nine CoCs embedded in leaked plastics increased from 0.30 Mt to 0.53 Mt, leading to the cumulative releases of 89.7 kt (95% CI: 42.4-259.7 kt) in the ocean by 2020. Their releases are highly concentrated in the tropical and subtropical regions of Asia, which accounted for 49.9% of the global mass. They are all classified as lower middle-income countries (LMCs). A cross-sectional analysis among countries reveals an inverted U-shaped relationship between the per-capita mass of CoCs embedded in leaked plastics and per-capita gross domestic product (GDP), consistent with the Environmental Kuznets Curve (EKC) hypothesis. The inflection point occurs at a per-capita GDP of approximately 2,208 dollars. By 2020, about 73.5% numbers of countries have surpassed this threshold. However, the decoupling currently stems primarily from improved waste management systems, rather than decrease in chemical use. The scenario analysis reveals that, relying solely on either reducing plastic consumption or optimizing waste management will partially curb the increase in the release of CoCs, while reducing chemical use will substantially mitigate the releases, with annual release peaking in 2071 before declining. The results highlight the importance of reducing use of CoCs to curb their long-term environmental impact in the ocean.

This study provides scientific evidence for understanding plastic pollution in the marine environment, and offers decision support for sound management of chemicals, particularly for the upcoming global plastics treaty.

Disclaimer/Disclosure:

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3.17.P-Tu321 Endocrine disruptive potential of a new bisphenol A substitute DD-70

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The increasing number of evidences about the endocrine disruptive effects of bisphenol A (BPA) have led to its replacement with insufficiently explored structural alternatives. DD-70 (1,7-bis (4-hydroxyphenylthio)-3, 5-dioxaheptane) belongs to the novel BPA substitutes mainly found in thermal paper. Having bisphenol structure, DD-70 could have the similar estrogenic profile as BPA. Hence, the aim of this study was to investigate the endocrine disruptive potential of this compound using in silico tools.

SwissADME was used for the calculation of molecular properties and the prediction of gastrointestinal absorption of DD-70 whereas GOLD software was applied for molecular docking. The binding affinity of DD-70 towards estrogen receptor alpha (ER α , pdb: 7UJO), beta (ER β , pdb: 5TOA) and gamma (ER γ , pdb: 6KNR) was evaluated in comparison to BPA using ChemPLP as a scoring function.

Based on the analyzed molecular properties, DD-70 met Lipinski's rule of five criteria for good oral bioavailability as well Veber's rule with predicted high gastrointestinal absorption. The obtained ChemPLP scores of DD-70 for ER α , ER β , and ER γ were 83.92, 71.04, and 73.28, respectively, in comparison to co-crystallized ligand scores of 99.97 (ER α), 67.46 (ER β), and 116.35 (ER γ). However, when compared with the ChemPLP scores obtained for BPA (ER α 60.36, ER β 55.54, and ER γ 59.83), DD-70 had much higher binding affinity. The obtained results suggest DD-70 high potential for impairment of normal estrogen signaling through the interactions with estrogen receptors.

In silico results imply health risks associated with the exposure to DD-70 as xenoestrogen. Both in vitro and in vivo studies are needed in order to better understand the endocrine disruptive potential of this new BPA alternative.

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3.17.P-Tu322 Persistent Organic Pollutant Signatures in Cory's Shearwater Exposed to Plastic Ingestion

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Plastic ingestion by seabirds is widely recognized as a physical threat, yet its role as a vector of organic contaminants remains insufficiently characterised under real environmental conditions. This study evaluates the occurrence and profiles of persistent organic pollutants (POPs) in Cory's shearwater (*Calonectris borealis*) in relation to previously and fully characterised plastic and microplastic loads detected in their proventriculus and ventriculus.

A subset of 57 fledglings stranded during rescue campaigns in Tenerife (Canary Islands, Spain) in 2022 and 2023 were examined and analyzed for POP concentrations in ingested plastics, liver, and, in some cases, adipose tissue. Up to 53 compounds were determined, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), by means of a validated analytical method based on ultrasound-assisted extraction, gel permeation chromatography clean-up and GC-qqq-MS/MS, with additional purification steps for tissues when required. The method showed recoveries of 60–95%, high reproducibility (RSD <8%) and limits of quantification below 1 pg g⁻¹ for most target compounds.

Concentrations were generally higher in plastic particles than in liver on a fresh weight (f.w.) basis, whereas values were comparable when normalized to lipid content. Distinct congener patterns were observed between matrices: plastics were dominated by PBDE-209 and lower-chlorinated PCBs, while liver samples showed prevalence of PCBs-153/138, PBDEs-47/99/100 and p,p'-DDE, reflecting selective bioavailability, transformation processes and additional dietary sources. Moderate monotonic correlations were detected between POP loads in plastics and ΣPCB concentrations in liver on a f.w. basis, but not when expressed on a lipid basis. Moreover, the total mass of ingested plastics showed a negative logarithmic relationship with ΣPCB concentrations.

These results support a multifactorial exposure scenario rather than a single direct transfer pathway from plastics to tissues. The combined assessment highlights the relevance of Cory's shearwater as bioindicator and provides robust evidence to inform integrated monitoring strategies addressing both plastic pollution and chemical contamination, particularly in the Canary Current ecosystem, an area of high ecological and socioeconomic importance.

3.17.P-Tu323 Distribution Characteristics and Risk Assessment of Phthalic Acid Esters in 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 long-term large-scale use of agricultural mulch film in Hetao irrigation district results in residues of plasticizers such as phthalic acid esters (PAEs) in the irrigation district, while pollution in groundwater caused by PAEs from agricultural mulch film was lack of systematic research. In order to investigate the distribution characteristics of PAEs in 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. Groundwater samples were collected before spring irrigation, after spring irrigation, before autumn irrigation, and after autumn irrigation, respectively, and PAEs in these samples were analyzed by gas chromatography-mass spectrometry. The results showed that the concentration of PAEs in these samples ranged from 0.14 µg•L⁻¹ to 9.80 µg•L⁻¹ during the two irrigation periods. Diethyl phthalate and dimethyl phthalate were the major constituents in groundwater during spring irrigation. Dibutyl phthalate and di(2-ethylhexyl) phthalate became the major PAE constituents in groundwater after autumn irrigation. The concentration of PAEs in groundwater increased by 20.3% after spring irrigation and by 115.5% after autumn irrigation. The infiltration of irrigation water containing PAEs into the groundwater environment is an important reason for the increase of PAEs concentration after irrigation. Iso-octyl phthalate was a major high-risk pollutant, and its annual risk factor value far exceeds the safety threshold, mainly due to its high lipophil and low degradation, leading to prominent bioaccumulation risk. The results will provide a cutting-edge research basis for preventing and controlling PAEs pollution in the irrigation district.

3.17.P-Tu324 Tire wear particles (TWPs) as a vector for chemicals from urban environments

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Tire wear particles (TWP) are formed through the abrasion of tire treads during driving. They represent a specific form of microplastic (MP) pollution and contribute to an essential portion of the overall MP emissions, with around 6 million tons of TWP emitted annually across the world and therefore ranked as the second most abundant source of primary MP to the world's oceans. Antioxidants and antiozonants play crucial roles in preventing degradation of the tire material during use. These additives can constitute between 5-10% of the weight of the tires. Paraphenylenediamines (PDs) are commonly used as additives with N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) being one of the most widely used PDs. These chemicals and their transformation products, especially PD-quinones, have been shown to be bioavailable and toxic to a range of aquatic organisms. Because of their complex composition, TWP not only disrupts natural systems as anthropogenic particles, but also as a source of harmful soluble additives. These additives create additional environmental and human health concerns due to their leaching potential.

Total deposition samples were collected during 2022 – 2025 at two remote locations (Svalbard, Zeppelin station and Birkenes station) and two urban locations (Oslo and Trondheim) in Norway. To evaluate the ability of TWP chemicals to be taken up by recipients of emissions to the marine ecosystem, a variety of marine species were sampled along sites with known TWP pollution as well as reference sites. In addition, exposure experiments in blue mussels, a commonly consumed seafood species, were conducted to better understand the uptake kinetics of TWP chemicals.

Based on our findings of TWP in urban deposition samples and marine species impacted by tire rubber pollution, evidence for both airborne as well as dietary human exposure to TWP and related chemicals were found in our study. Bioavailability of selected TWP chemicals, the PDs, could be shown in both exposure experiments as well as field samples. Elimination capability of PDs exposure seems to vary between marine species, posing unknown longterm risks together with potential dietary exposure risks to humans. For additional human health considerations, especially urban settings, with a high occurrence of atmospheric TWPs can be potential high exposure risk to both PM10 sized (and smaller) particles and related chemicals.

3.17.P-Tu325 Quantification of Tyre Wear PM10 Microplastics From Recycled Rubber Flooring With Pyrolysis-Gas Chromatography Coupled With Mass Spectrometry (Py-GC-MS)

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Non-exhaust vehicle emissions are the main source of tyre wear microplastics, mainly made of natural rubber (NR) and artificial rubber, its main constituent being styrene-butadiene rubber (SBR), but recent years have seen a growing concern regarding other sources, like the photooxidative, chemical and mechanical wear of recycled rubber surfacing, found in playgrounds and sport facilities. Due to the proximity and physical activity of pitch users in sport facilities, and the higher effect of exposure for children in playgrounds, these sources represent an acute and significant exposure to tyre wear microplastics to their users.

On top of the known challenges microplastic analysis poses, like difficulty to isolate from the environment and low analytical selectivity for most methods, tyre NR and SBR are subjected to a vulcanization process during synthesis, binding polymer strands with sulphur bonds to give these materials the durability tyres require. Vulcanization makes these microplastics remarkably inert, making their analysis more challenging, and requiring long and complex sample treatment.

To bypass this issue, a new method was developed, consisting in pyrolysis-gas chromatography coupled to mass spectrometry (py-GC-MS). The high temperature of the pyrolysis furnace is able to decompose vulcanized rubber material back into its oligomeric constituents with minimal sample treatment, thus providing a simple and reproducible analytical method for NR and SBR monitoring.

8 sampling sites were studied in the city of Barcelona, where deposited PM10 was sampled using a portable vacuum sampler with separation chamber in PM10-selective regime. Suspended PM10 was also studied in one sampling site, using PM10-selective air pumps. Corresponding field and method blanks were taken and analysed as well. A py-GC-MS method was optimized for the analysis of NR, also known as polyisoprene, and SBR, using selective markers for quantitation and internal standard calibration. Analysis of mock samples was made to ensure the devulcanization of rubber microplastics under pyrolysis and acceptable recoveries.

Results show NR and SBR have been detected in deposited PM10. They were, however, not detected in suspended PM10 significantly above blank samples. Results prove the method's effectiveness in detecting tyre wear microplastics, simplifying the complex analytical problem vulcanized rubber poses when using conventional sample treatment and extraction.

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3.17.P-Tu326 Trace elements in plastics – an environmental case study of sorption and release

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Global plastic pollution poses a major environmental challenge. An often-overlooked danger lies in the chemical additives of the plastics. A variety of additives are mixed into plastics during manufacture to give them specific properties such as color, stability, or flexibility. However, these can be released from the material under environmental influences such as UV radiation and/or contact with water. To understand the extent of this problem and to enable sound risk assessments, it is crucial to investigate the behavior of additives under real environmental conditions.

Therefore, we deployed plastic sheets made from widely used polymers (PE, PET, PVC) with defined additives containing elements like Mg, Ca, Zn, As and Sb, at a research platform in the North Sea. Polymer plates with defined mass fractions of target metals were custom made by SKZ – Das Kunststoff Zentrum (Würzburg, Germany). The plates were deployed between December 1, 2023, and November 20, 2024, at a research platform in Cuxhaven (Germany). Polymer types included with known amounts inorganic and organic additives. Samples of the polymer plates were taken after 7, 15, 22, 30, 36 and 51 weeks.

After deployment approx. 200 mg of the plates were acid digested using a microwave-assisted acid digestion (MWAD) protocol (6 mL HNO₃ and 2 mL HCl; 220 °C). Quantification of 55 elements was performed using an ICP-MS/MS system (8800 Triple Quadrupole ICP-MS, Agilent Technologies, Tokyo, Japan). The analytical process was validated using the reference materials NMIJ CRM 8123-a (PVC), NMIJ CRM 8133-a (PP), BAM-H010 (ABS), ERM-EC680m (PE) and ERM-EC681m (PE).

Depending on the polymer type and element different sorption/desorption behaviors were observed. We observed mainly sorption of selected elements like Mn and Fe to the surface likely caused by precipitation and or biofilm growth. For elements with high mass fractions like Sb in PET or Zn in PVC that were added during the manufacturing process, leaching effects could be observed. These elements are of special interest, as they are also highly abundant in consumer plastics due to their usage as catalyst (Sb in PET) or additive (Zn-stearate in PVC).

This pilot study highlights the complex nature of sorption and desorption processes of inorganic elements from custom made plastics under real world conditions. Understanding these processes is key to mitigate the environmental impact of plastic products during their lifetime and following disposal.

3.17.P-Tu327 Assessing the Environmental Persistence of Commercial Phenolic Antioxidants

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Synthetic phenolic antioxidants are widely used as plastic additives to maintain polymer integrity under oxidative stress. Despite their extensive use and frequent detection in various environmental matrices, data on their environmental fate and (eco-)toxicity remain limited. This work aims to address this hazard assessment gap by investigating the environmental persistence and sorption behaviour of 60 commercially available phenolic antioxidants using activated sludge biotransformation assays.

To this end, a targeted ESI-LCMS method was developed to quantify dissolved and total concentrations in activated sludge. Compounds were tested in mixtures at environmentally relevant concentrations, and biotransformation tests were conducted over 72 hours. By analysing both supernatant and ethanol-extracted sludge samples, the extent of partitioning between the dissolved phase and the activated sludge was assessed. Observed biotransformation rate constants of parent antioxidants were determined assuming first-order degradation kinetics. The experimental data collected will be used to further infer sorption coefficients and biomass- and bioavailability-corrected biotransformation rates.

Observed biodegradation in activated sludge depends on both the compound's intrinsic biodegradability and its bioavailability to the microbial community. Across the selected set of antioxidants, some compounds exhibited very low dissolved fractions, indicating that adsorption to the sludge flocs strongly influences their fate in activated sludge. For those antioxidants, quantifying the partitioning between the dissolved phase and the activated sludge is essential for interpreting the results of biotransformation experiments. Therefore, the efficiency of the sludge extraction procedure was assessed for all selected antioxidants, yielding a mean extraction efficiency of 75%. For antioxidants with higher bioavailability, observed biodegradation rate constants were derived based on dissolved concentrations.

Overall, this study fills an important knowledge gap in the environmental hazard assessment of synthetic

phenolic antioxidants. The testing workflow and the generated data can be integrated into early stages of the chemical development process for new, emerging antioxidants, thus providing essential information for designing safe and sustainable chemicals and materials. Future work will focus on coupling activated sludge biotransformation assays with in vitro (eco-)toxicity assays.

3.17.P-Tu328 Analysis of Antioxidants and UV Absorbers in Forty-Four Recycled Plastic Pellets

Naohide SHINOHARA, Naoya KOJIMA, Tomoko OGURI, Kyoko ONO, Isamu OGURA and Masashi GAMO, National Institute of Advanced Industrial Science and Technology, Japan

Introduction

To achieve a low-environmental-impact circular plastic economy, expanding the use of recycled plastics is essential. However, their safety has not been fully evaluated, particularly regarding residual or unintentionally introduced additives. Antioxidants and UV absorbers prevent thermal and photodegradation, but some persist, migrate, or show toxicity. Yet, unlike flame retardants and plasticizers, information on them remains limited. This study quantified these additives in recycled polyethylene and polypropylene pellets.

Methods

Eight antioxidants and eight UV absorbers were analyzed in 44 samples of recycled plastic pellets collected from 10 recycling plants in Japan. Approximately 200 mg of pellets were treated with 10 mL of cyclohexane at 150 °C for 4 h in a high-pressure vessel to extract additives. After centrifugation, the supernatant was filtered and concentrated to 1.0–1.5 mL by nitrogen blowdown at 30 °C, then diluted to 10 mL with methanol to reprecipitate the resin. The solution was centrifuged, filtered, concentrated to ~1.0 mL, internal standards were added, and the volume was adjusted to 3.0 mL with dichloromethane. The extracts were analyzed using GC–MS.

Results

The most frequently detected antioxidant was pentaerythritol tetrakis[3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate] (Irganox 1010), found in 98% of samples (<0.3 to >403 µg/g). Octadecyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (Irganox 1076) was detected in 39% (<2.0 to >265 µg/g). 2,4-Di-tert-butylphenol (Prodox-146) and butylated hydroxytoluene (BHT) were detected in 34% (<0.3–39.3 µg/g) and 14% (<0.3–83.2 µg/g), respectively. Among UV absorbers, UV770 was detected in 6.8% (<3.5–76.5 µg/g), while UV327 and UV329 were each observed in only one sample (48.6 and 1.14 µg/g, respectively).

Discussion

Because the EU Persistent Organic Pollutants Recast Regulation will restrict UV328 in recycled products to <1 µg/g as an unintentional trace contaminant (UTC) by August 4, 2029, all analyzed pellets were within this requirement (all samples were below the limit of quantification: 0.3–0.7 µg/g). Other UV absorbers of regulatory concern, such as UV-329, UV-320, and UV-350, are listed as Substances of Very High Concern (SVHC) in Europe and might be subject to future restrictions; however, only a small number of samples contained detectable amounts. In contrast, the antioxidants were detected more frequently and at higher concentrations than the UV absorbers.

Disclaimer/Disclosure:

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3.18.A Microplastics Research: Beyond Fear-Mongering, Towards Trustworthy Science

3.18.A.T-01 Addressing Matrix Effects in Micro- and Nanoplastic Quantification Using Pyrolysis-GC-cIMS-HRMS

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Studies reporting the presence of micro- and nanoplastics (MNPs) in human biological samples are increasing, raising concerns about exposure and potential health effects. Current exposure assessment commonly relies on

pyrolysis coupled to gas chromatography – mass spectrometry (Py-GC-MS), where quantification is based on the detected abundance of polymer-specific markers. However, a key limitation of Py-GC-MS is its potential for polymer overestimation when non-plastic matrix components form identical markers during pyrolysis. Such interferences complicate toxicological interpretation and contribute to public concern. At the same time, quality assurance and control practices to mitigate such misidentifications are not consistently implemented, making the reliability of reported exposure data uncertain.

A recent advancement in the quantification of MNPs is the use of cyclic ion mobility spectrometry (cIMS) hyphenated to high-resolution MS (HRMS). The improved separation and resolution of Py-GC-cIMS-HRMS provide additional analytical specificity by reducing spectral background, thereby preventing false-positive detections. In this study, we applied a non-target approach to identify MS features that uniquely correlate with the polymers PE, PP, PS, PET, PVC, and PMMA. Across all samples, peak picking yielded 35,365 MS features for which linear calibration models were fitted. Based on each model's coefficient of determination (R^2), for each polymer optimal markers were identified that remained robust in the presence of sample matrix.

Our preliminary findings indicate that PE quantities can be overestimated when commonly used short-chain α -alkene markers are employed, whereas long-chain α -alkenes or α,ω -alkadienes remain robust with limited matrix effects. For PET, potential underestimation may occur due to co-pyrolysis with matrix residue. The stronger matrix effects observed here compared to earlier reports likely result from the use of single-shot pyrolysis, the standard approach for MNP quantification. Instead, in double-shot pyrolysis, volatiles are removed prior to polymer pyrolysis, thereby reducing the formation of nonspecific fragments such as α -alkenes and limiting co-pyrolysis with more reactive polymers like PET. Together, these results demonstrate the value of Py-GC-cIMS-HRMS for improving marker selection and strengthening the reliability of MNP exposure assessments.

3.18.A.T-02 Best practices for the detection and quantification of micro- and nanoplastics in biological tissues

Willie Peijnenburg, University Leiden, Netherlands

Micro- and nanoplastics (MNP; 1 nm–5 mm) have become a major environmental and public-health concern. While numerous studies have documented their occurrence in various ecosystems and biological matrices, methodological inconsistency continues to undermine data comparability and risk interpretation. This contribution presents an internationally validated framework of 28 best practices for the detection and quantification of MNP in biological tissues. Developed through an expert-reviewed consensus process, this framework represents a globally harmonized foundation for methodological reliability.

Each recommendation was derived from a systematic review of 46 interlaboratory comparison studies, validated by independent experts, and grouped into four pillars: Quality Assurance and Quality Control (QA/QC), Sample Collection, Particle Quantification and Qualification, and Data Handling and Reporting. Although the primary focus lies on biological tissues such as blood, muscle, and organ samples, the same principles apply broadly to environmental and industrial matrices. The aim is to increase reproducibility, minimize contamination, and enable transparent cross-study comparison.

3.18.A.T-03 Identification of Unknowns using Vibrational Spectroscopy – A Case Study of Microparticles in the Environment

Matthew Wagner, The Procter & Gamble Company, United States

The rapid expansion of micro- and nanoplastic (MNP) research has created a need for robust analytical methods to ensure the accuracy of findings. This presentation reviews analytical approaches for identifying environmental MNPs, using vibrational spectroscopy as a case study to highlight common challenges and best practices. While techniques like Fourier transform infrared (FTIR) and Raman spectroscopy are powerful tools for chemical identification, over-reliance on automated software and Hit Quality Index (HQI) metrics for spectral matching can lead to misidentification. A case study demonstrates how different software packages can yield conflicting results for the same material, underscoring the critical role of the experienced analyst in interpreting spectral data. Accurate identification in complex environmental matrices requires a commitment to analytical rigor, including thorough sample preparation, an awareness of spectral library limitations, and the application of complementary, multimodal analytical techniques to confirm findings. Advancing the scientific understanding of MNPs demands a holistic approach that goes beyond advanced instrumentation to include improved data analysis, a critical evaluation of results, and the development of standardized methods to enhance data reliability.

3.18.A.T-04 Reliable Riverine Microplastics Monitoring: Improvement and Harmonization of Sampling, Analysis and Reporting

Guidrun Obersteiner, Reinhold Ottner, Sabine Lenz and Christian Zafiu, University of Natural Resources and Life Sciences (BOKU), Austria

Despite growing attention to microplastics, methods for detecting them in river ecosystems remain heterogeneous. Differences in sampling, preparation, and analysis make comparability difficult. Partners from seven countries in the Danube basin worked to improve and harmonize existing monitoring approaches to enable reliable statements about the distribution of microplastics.

Since varying infrastructure of participating organizations, a common methodological framework was developed, tested, and adapted to local conditions. Two comparative sampling campaigns provided important insights into the handling of different pump and net systems and enabled harmonized procedures on site. Standardized protocols ensured that all partners recorded comparable metadata such as flow velocity and hydrological conditions. Pre-treatment and analysis were carried out according to sample type, laboratory equipment, and budget. Various sample splitting methods were tested for representativeness.

Scientifically, a combination of pump and net sampling is recommended, as both methods cover different particle size ranges and can be used simultaneously. This allows a representative water volume to be recorded and different analysis parameters (mass, particle count, polymer type) to be addressed flexibly. Standardized application improves comparability, supports the identification of hotspots, and provides a basis for effective mitigation measures. The results show that analytical methods—especially FTIR methods and databases used—can have a significant impact on particle counts.

The following key challenges were identified: representative sampling, harmonized metadata collection, standardized pretreatment and analysis, uniform reporting, and quality assurance.

These aspects are taken into account to formulate standardized protocols. Complete standardization remains difficult due to varying resources and issues. In the future, however, key parameters like mandatory size fractions should be introduced. In addition to the particle count, the determination of mass as a stable parameter is particularly relevant above 500 µm. A new measurement parameter focussing on the area of plastic could also be introduced, especially for analysis methods where the plastic particles are scanned over a large area, in order to avoid overestimating the particle count. Interlaboratory studies and suitable reference materials are urgently needed to improve the comparability of polymer identifications.

3.18.A.T-05 Index-based Environmental Risk Assessment of Micro- and Nanoplastics: Handbook for Conscious, Accurate, and Standardized Use

MANUELA PICCARDO and Stanislao Bevilacqua, University of Trieste, Italy

Pollution from micro- and nanoplastics currently represents one of the main environmental challenges, raising concerns both for its ecological consequences and for the potential risks to human health. After two decades of data collection since the first appearance of the term “microplastic” in the scientific literature, research is moving toward a new direction — that of assessing ecological and human health risks. In this perspective, a rising interest is converging on the use of indices, which may represent an effective tool for describing environmental quality in a clear and straightforward manner.

This contribution, based on the systematic review of 316 papers collected from the Web of Science database, aims to examine the use of indices for environmental risk assessment from both an editorial perspective (when the first publication dates back to, how the topic has evolved over time, and which publishers and journals have shown the greatest interest) and a technical point of view (which and how many indices have been proposed to date, which environmental matrices have been considered, and which geographical areas have received the most attention). Furthermore, what limitations does each index present, and how can they be improved? The goal is to gather and summarize all information on the topic, which to date remains somewhat fragmented, and propose a roadmap to guide researchers and practitioners through an informed, accurate, and standardized use of indices for ecological risk assessment of microplastic pollution.

3.18.B Microplastics Research: Beyond Fear-Mongering, Towards Trustworthy Science

3.18.B.T-01 Distinguishing the Aqueous behaviour of Water Soluble Polymers from Nano-plastics with Advanced Analytical Techniques

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The widespread use of the term “nano-plastics” risks implying every polymer to be classed as a nano-plastic, despite general academic consensus and adoption into regulatory frameworks of micro/nano-plastic definitions.

This risk is acute with water soluble polymers. A single macromolecule chain of a water soluble polymer is nano-sized in solution, but does it behave the same properties as a “small nano-sized microplastic” dispersed in water? This is important for classifications, and to ensure the right methods are applied to the relevant species to assess environmental risk.

Water soluble polymers have been studied for a number of years to determine their water binding preferences. Environmental aspects of such polymers is now also in focus.

We studied Polyethylene Glycol (PEG), Polyvinyl Alcohol (PVA) (across grades of solubility) and Polystyrene (PS). From simple filtrations, we have expanded to the aqueous nanoscale using Small Angle X-ray Scattering (SAXS), Dynamic Light Scattering (DLS), and Atomic Force Microscopy (AFM). The resultant structures show different single macromolecule conformations (for soluble materials), or hard spheres. It validates prior academic work using other techniques. The structures seen give insights into predicted environmental fate, eg contaminant adsorption or degradation.

We are extending the techniques to study if water soluble polymers could exhibit nano-plastic characteristics in the aqueous environment. From simple filtration experiments, we expanded to SAXS and DLS to look at individual or mixed water soluble polymers in various simulated environmental conditions. With complex systems, including mixture with surfactants, low water environments, we developed GPC and NMR to study hydrogel formation and solubilization behaviour. Results will be shared from our recent work, showing when formation of nano/microplastics is possible.

This work expands the toolbox to understand how water soluble polymers behave in water compared to nano-plastics. They demonstrate difference in solution conformation depending on structures or conditions. It can also provide insights for regulators to classify polymers as soluble or nano-plastic, to ensure subsequent application of the correct risk assessment procedures.

3.18.B.T-02 Beyond Size and Shape: Characterising Physicochemical Properties of Microplastic Test Materials Generated by Cryomilling

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Cryomilling is a widely adopted method of generating microplastic test materials for environmental studies: however analysis of these test materials is often limited to size and shape, and it is assumed other physical, chemical and thermal properties are preserved. Here, this assumption is challenged to provide insight into the effect of cryomilling on physicochemical properties which can influence microplastics behaviour and impact.

A comprehensive analysis of several virgin polymer pellets, including low-density polyethylene, polypropylene, polylactic acid and polybutylene succinate, and a weathered agricultural mulch-film, was performed before and after cryomilling. Scanning electron microscopy and particle size analysis showed that particle size and shape were influenced by milling parameters, such as chamber size, and polymer type. Surface-specific additives identified by FTIR spectroscopy were redistributed throughout the milled material, but weathering-induced changes were preserved. Bulk polymer chemistry was maintained after milling, confirmed by pyrolysis gas-chromatography mass spectrometry. However, polymer-specific changes in crystallinity were demonstrated using thermogravimetric analysis with differential scanning calorimetry. Contamination from metal and plastic milling components was concluded to be minimal. These results show that it cannot be assumed that physicochemical characteristics are preserved during cryomilling and therefore should be considered when characterising microplastic test materials used in environmental studies. Furthermore, characterising these materials beyond size and shape allows for a more realistic comparison between microplastic test materials and environmental plastics.

3.18.B.T-03 The Plastic in Microplastics

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Microplastics (MPs) are tiny synthetic particles ranging between 5 mm and 1 µm. Each particle can consist of one of a variety of synthetic materials, produced from a series of polymer chains and chemical additives, to impart the characteristics needed for the final use of each plastic material. This leads to particles having unique characteristics between polymers and within each polymer. When these particles enter the environment, they undergo further physical and chemical alterations. Surface fracturing, volume and density changes, biofilm growth, and the ability to release and absorb toxicants from the surrounding environment lead to a scenario in which each MP particle may have unique traits and how they could impact organisms. These points lead to particles that are difficult to sample, analyse, characterise, and assess their impact on biota. In this study, MPs in rivers and streams in the Autonomous Province of Bolzano were investigated. Ten sites were selected where nine replicate sediment samples were collected to determine the MP content. These consisted of three bank replicates, three instream at 20 cm deep, and three instream at 5 cm deep to report a more representative abundance of MPs. The sediment was then dried, and MPs were separated through density separation with ZnCl₂ and centrifugation. Particles were then filtered, quantified through stereomicroscopy, and characterised with a Shimadzu ATR-FT-IR with Labotech software and compared to spectra of both organic, additive, and polymer libraries. The mean concentrations varied between 38.85 ± 22.23 particles per kg in spring and 32.59 ± 10.94 particles per kg in autumn. Concentrations ranged between 16.65 and 79.55 particles per kg in the spring and 12.96 and 46.29 particles per kg in the autumn. Polymer analysis found that Polyester was the most prevalent polymer type. Other materials included polypropylene, polyacrylic, and other artificial natural fibres such as Bemberg. Some chemical additives were also identified; however, these can only be hypothesised, with more formal analysis required to concretely determine the type and concentrations of each additive present. Fifty additive signatures were detected, including 16 which are described as toxic to the aquatic environment, and more than half with no toxicological data available. This highlights not only the importance of studying MP abundances, but also the additives and other toxicants which may be bound to these particles.

Disclaimer/Disclosure:

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3.18.B.T-04 Towards Standardized and Open Data Reporting on Litter Monitoring in Inland and Transitional Waters

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Litter surveying and monitoring in the aquatic environment still relies on disparate reporting methodologies. While the Marine Strategy Framework Directive (MSFD) provides guidance for marine environments, comparable requirements are limited under the Water Framework Directive (WFD) and have not been implemented yet, resulting in heterogeneous datasets and reduced comparability across studies. To address this gap, we developed FAIR-compliant and openly accessible dataset templates for micro-, meso-, and macrolitter monitoring that align with existing international best practices for gathering and managing metadata and data, including the guidelines of the European Marine Observation and Data Network (EMODnet). A structured development workflow was followed, informed by European directives, technical guidance documents, and controlled vocabularies. The templates were tested using pilot datasets and refined through multiple local and European projects (e.g., PLUXIN, PLASTFLOW, TREASURE, INSPIRE) to ensure applicability across diverse sampling environments and methods. Delivered in a machine-readable format (*.CSV), the templates capture campaign-level metadata (e.g., geographical and temporal coverage, environmental setting, and responsible institutions) as well as detailed methodological information such as sampling approach, equipment, and processing workflows. Litter observations are reported at the sample or item level, with standardized descriptions of material, size, count, morphology, and other key characteristics. These harmonized templates

enhance transparency, interoperability, and long-term reusability, enabling more reliable spatial and temporal assessments of litter pollution in aquatic environments. By translating policy needs into practical data structures, this work supports a consistent, FAIR, and open workflow for litter monitoring and reporting in inland and transitional waters.

3.18.B.T-05 Do Current Microplastic Data From Global Karst Groundwater Show Real Environmental Contamination or Methodological Bias?

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Microplastics have been found in karst groundwater in several regions, but the overall picture is still unclear. Most studies come from only a few countries and focus on caves, whereas springs and wells in many karst areas remain unexplored. Reported concentrations vary from low to extremely high, but it is not always clear how much of this variation reflects the hydrogeology, contamination sources and how much comes from the methods used.

To clarify this, we compiled published studies on microplastics in karst groundwater and examined how methodological choices shape the results. We compared the main approaches used for sampling, particle extraction and identification, and considered how these choices affect what each study detects. Variation in detection limits, polymer confirmation methods, and contamination control helps explain the high variation in number of particles detected between studies. For instance, Raman or micro-Fourier Transform Infrared Spectroscopy detect many more small particles and a wider range of polymers, while studies that rely mainly on visual identification using microscopy or sodium chloride for density separation are biased toward larger or low-density particle fragments and fibres.

To assess how comparable the available data are, we applied existing quality assessment criteria for microplastics. This helped show where analytical biases are likely to happen and where results are better supported by stronger QA/QC. It not only helps us identify uncertainties but also shows where studies are directly comparable despite using different methods. Although some studies report using basic contamination control and limited spectroscopic confirmation, recovery tests are never carried out, and visual identification is still common practice, restricting the types of microplastics that can be confidently identified, typically towards larger plastic fibres.

By putting the published evidence into context, we show that many of the differences between studies reflect methodological bias rather than differences in environmental contamination. By linking the results published so far to their sampling and analytical choices, it becomes clearer where studies can genuinely be compared and where methodological artefacts limit what can be concluded. This supports more realistic communication of microplastic exposure in karst groundwater and highlights the benefit of moving towards more consistent and standardised approaches in future work.

3.18.P Microplastics Research: Beyond Fear-Mongering, Towards Trustworthy Science

3.18.P-Mo190 Beyond Polystyrene: Precipitation-Based Production of Pristine Micro- and Nanoplastic (MNP) Test Materials

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Polystyrene remains the dominant focus of ecotoxicological micro- and nanoplastic (MNP) research, despite representing only a minor share of global plastic production. Environmentally relevant test materials from polymers such as LDPE, PET, PA6 and PP remain scarce, particularly below 20 µm.

A fast and straightforward precipitation-based approach is presented in this work for producing pristine MNP reference materials from these polymers using tailored solvent–nonsolvent systems. The method enables the generation of irregular and spherical particles within a reproducible micro- and nanoplastic size range, yielding LDPE (0.5–10 µm), PET (0.4–4 µm), PA6 (0.6–6 µm) and PP (0.2–6 µm). Polymer-specific yields of 250–920 mg of MNP material can be achieved in a single run.

Accelerated UV ageing was applied to mimic environmentally relevant processes, with the precipitated particles being photo-oxidized and subsequently characterized by FT-IR and zeta-potential measurements. Pristine particles showed strong agglomeration tendencies, whereas the UV-aged materials exhibited enhanced colloidal stability in aqueous systems, with zeta potentials below -30 mV at pH 7, significantly reducing their propensity to cluster. A concept for producing pristine MNP particles containing polymer additives is furthermore introduced.

This study ultimately provides two key advancements: (1) a precipitation-based route for generating polymer-diverse reference materials for MP/MNP characterization, and (2) an ageing procedure that enhances environmental relevance by modifying particle surface chemistry and dispersion behavior.

3.18.P-Mo192 Towards fit-for-purpose monitoring of microplastics

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The past 20 years of research has left us in no doubt that microplastics are ubiquitous across the globe.

However, whilst we are not lacking in data, we are limited in generalising insights from the extensive observational studies to date. Uncertainty is inherent to the measurement of any chemical. Whilst not unique to microplastic research, it is proving particularly challenging when communicating the risks of microplastic emissions. Standardisation of measurements is often cited as a solution. However, standardisation can only occur once the field has matured sufficiently to guide which of the available methods can deliver relevant data and meet the needs of the many stakeholders who will rely on these measurements.

Many approaches have been used to measure microplastics in the environment. These range from the relatively cheap, looking for easy to spot large particles, to more expensive analyses, targeting challenging particles only a few microns in size, or identifying polymers themselves. No single method is quantitative of microplastics across their entire diversity of size, shape and chemistry. However, if we consider specific monitoring scenarios, we can begin to identify and develop methods which are “fit-for-purpose”.

Any candidate method consists of a series of steps: sample collection, preparation, analysis and interpretation. There is variation associated with each of these steps, which in combination establishes the total uncertainty. When considering wide scale monitoring, the suitability of candidate methods can be considered a balance between the accuracy and precision of the data generated (the “uncertainty”), versus the costs, throughput and readiness of these methods for deployment at scale.

Here, we present a framework to identify fit-for-purpose monitoring for microplastics (UKWIR, EQ/01/A/279). Taking the hypothetical scenario of national monitoring for microplastics in wastewater effluent, we show how uncertainty, sample number, confidence and costs are linked and can be combined in an evaluative framework, to select suitable methods to develop or deploy at scale. This framework would allow candidates to be ranked, cost efficiency to be considered and recommendations for targeted research to reduce uncertainty or validate specific elements of the method(s) to be identified. In this way, the framework acts as a harmonised, structured approach, applicable to many scenarios, to support the development of fit-for-purpose monitoring.

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3.18.P-Mo194 Assessing Sampling Effort Needed to Detect Microplastic Declines in the Scheldt Estuary for Policy Support

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Monitoring plastic pollution in estuarine environments is complicated due to high natural variability and multiple interacting physical, chemical, and biological processes affecting transport and retention. In the Scheldt

Estuary in Belgium, tidal dynamics, freshwater discharge, rainfall, sediment resuspension, vegetation, organic matter, and industrial activity introduce variability that can obscure temporal trends. Historical accumulation and burial-resuspension cycles of older plastics further complicate distinguishing new pollution. Following recent policy measures to reduce microplastic emissions, the PLASTFLOW project (2024–2025) aimed to update microplastic abundance estimates and evaluate the sampling effort required to detect policy-driven changes. Building on a 2020–2022 baseline study, sampling campaigns were conducted across eight estuarine sites during different seasons, collecting surface and water-column samples at multiple depths. Preliminary results confirm previously identified accumulation zones, particularly upstream of Doel and in the Antwerp region, with surface concentrations ranging from 25–28 particles/m³ and water-column concentrations from 93–122 particles/m³. Downstream locations generally exhibited lower concentrations, though Zeebrugge showed elevated water-column levels likely influenced by coastal inputs or dredge disposal. To inform monitoring design, a power analysis is currently being performed using observed variability to determine the number of samples per estuarine segment and season needed to detect policy-relevant changes ($\alpha = 0.05$, power = 0.8). Initial observations suggest substantially higher sampling intensity than currently implemented is required, which emphasizes the influence of both spatial and temporal variability on monitoring outcomes. By combining updated concentration data with statistical power assessment, PLASTFLOW will provide guidance for robust long-term monitoring frameworks, enabling reliable evaluation of mitigation efforts under the EU Green Deal's Zero Pollution Action Plan.

3.18.P-Mo195 Minimizing Biogenic Interference in Microplastic Quantification: Evaluation of Digestion Protocols and Counterstaining Strategies

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Microplastics characterization in organic waste remains a major analytical challenge due to the complexity of the matrix. The main issues arise from the high loads of biogenic materials, which can persist through multiple sample preparation steps. While chemical digestion has been studied to reduce organic content in various environmental matrices, complete removal has not been achieved without compromising polymer integrity. Residual natural particles can exhibit optical properties similar to those of plastics, leading to a substantial overestimation during microscopic-based quantification. In this study, we evaluated the efficiency of multiple digestion protocols for organic waste and introduced a subsequent two-step staining procedure to better visualize and distinguish microplastics from inorganic and biogenic particles. Since Nile red can fluorescently stain both plastic and certain natural particles, a counterstaining step using a water-soluble dye was introduced to mask and suppress the fluorescence signal from the biogenic interferences. The proposed workflow aims to minimize matrix-derived interferences and improve the reliability of microplastic analysis in organic waste.

3.18.P-Mo196 Development of a Portable Hollow-Fiber System for Sampling and Quantifying Microplastics Larger Than 1 µm

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Microplastics (MPs) in treated drinking water have emerged as a regulatory concern, particularly in the European Union where routine monitoring is mandated, and in California where tap-water monitoring guidelines have recently been proposed. Although these frameworks outline recommended sampling and analytical approaches for both raw and treated water, their implementation remains impractical. Current protocols require prolonged sampling and the use of large, costly concentration systems capable of processing several hundred liters of water in situ, presenting significant technical and logistical barriers. Moreover, multiple studies suggest that MPs detected in tap water may originate from post-treatment contamination within distribution systems rather than from water purification facilities, underscoring the need for point-of-use measurements. To accurately evaluate human exposure and associated health risks, reliable and scalable methods are needed to quantify MPs—including their concentrations, size distributions, and polymer chemistries—directly at consumer endpoints.

Here, we present a newly developed portable hollow-fiber concentration system (Mitsubishi Chemical Cleansui Corporation) designed for rapid sampling of MPs >1 µm in tap water. The system is compact and installation requires only attachment to a standard faucet, comparable to operating a household shower head. Sampling several hundred liters of tap water can be completed within 30 minutes automatically, and MPs are retained within a dedicated concentration chamber integrated into the shower-head unit. The retained fraction is subsequently digested using 30% hydrogen peroxide and further concentrated onto a 1 µm silicon membrane filter. Identification and quantification are performed using micro-Raman spectroscopy coupled with imaging-based particle recognition.

This presentation will demonstrate the system's contamination control and will report an example of measured MP concentrations, size distributions, and polymer compositions from a faucet. The results highlight the capability of this field-deployable method to enable scalable, regulatory-relevant monitoring of microplastics in drinking water at the point of human exposure.

3.18.P-Mo197 Validation of a pyrolysis - gas chromatography – mass spectrometry method for the determination of microplastics and its application in an interlab study

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Micro- and nanoplastics (MNP) are of health and environmental concern but little is known about the extent of the problem (1). Analytical techniques to reliably quantify MNP in environmental matrices are still under development. This is crucial to understand the concentrations we are exposed to and to devise mitigation strategies. Thermoanalytical techniques quantify MNP from nano- to millimeter size (2), however they are prone to disturbances from matrices, and the effect of polymer composition and weathering are not yet understood. These disturbances and uncertainties can lead to unreliable results causing misjudgment of human exposure.

In the pyHarmony project, which is part of the MOMENTUM consortium, we are developing reliable methods and performing interlab studies to gain knowledge on these topics. In this study, different commonly occurring MNP, i.e. polyethylene (PE), polypropylene (PP), polystyrene (PS), polymethylmetacrylate (PMMA), polyvinylchloride (PVC), polyethylene terephthalate (PET), nylon6 (N6) and styrenebutadienerubber (SBR) using the Frontier standard mixture were investigated. A pyrolysis GC-MS method was developed and validated and applied to fiber material from a washing machine filter, sand and river sediment samples spiked with PE, PP, PS and SBR from the interlab study. For all MNP three indicator pyrolysis products were found and limits of detection were below 1 ng absolute in all cases, with R2 values ranging between 0.990 and 0.999, recoveries between 80% and 120%, repeatability <20% and reproducibility <30%. With the optimized py-GC-MS method, PE, PP, PS and SBR were successfully identified and quantified in the sand and sediment samples and PET was identified in the fiber sample.

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3.18.P-Mo198 Adapting Raman microspectroscopy to different analytical aims for detecting microplastics in biological systems

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Plastic pollution represents a pressing environmental challenge. Micro/nanoplastics (MNPs) can form through environmental fragmentation or be intentionally produced, subsequently leaking into the environment. MNPs infiltrate food, water, and air, and can enter the human body through ingestion and inhalation. Understanding whether these particles are absorbed into the bloodstream and whether they reach specific tissues is critical for predicting toxicity.

Detecting and identifying MNPs in biological samples remains technically challenging due to their low concentrations and biological interferences. Raman microspectroscopy offers a label-free method for chemical imaging, enabling detection of submicron particles that are more likely to cross biological barriers and accumulate in human tissues.

This work explores how Raman microspectroscopy can be adapted to different analytical objectives—quantification, localisation, and biochemical assessment—across distinct biological systems. Three experimental examples illustrate the analytical trade-offs associated with each objective.

In the first example, quantification of MNPs in human blood focuses on identifying MNPs in digested samples, where low particle concentrations require matrix removal and concentration steps. The method was applied to

the analysis of blood from healthy donors ($n = 5$). Several polymer types were detected at low counts (< 10) and were absent in procedural blanks, suggesting their association with the blood matrix.

In the second example, cell culture experiments using THP-1 macrophages exposed to polyethylene terephthalate and polyamide MNPs demonstrate that Raman microspectroscopy enables localisation of particles inside cells.

In the third example, analysis of human tissue samples addresses both localisation and quantification. Here, a combined approach of digestion and cryosection analysis was applied to evaluate the feasibility of detecting MNPs within complex tissue structures.

Together, the results highlight how improving the likelihood of detecting MNPs often requires sacrificing spatial information about their location within the biological matrix. Rather than a single workflow, effective MNP detection requires tailoring Raman protocols to the specific biological question—whether quantification, localisation, or biochemical characterisation. Overall, this work defines methodological boundaries and optimisation strategies for Raman-based MNP detection in biological samples.

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3.18.P-Mo199 Advancing the Analysis of Micro- and Nanoplastics Using Multimodal Sub-Micron Optical-Photothermal Infrared (O-PTIR) and Simultaneous Raman Microscopy with Co-located Fluorescence

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Detection and characterization of microplastics (1 μm to 1 mm) and nanoplastics ($< 1 \mu\text{m}$) are crucial to monitor their prevalence and determine potential threats caused due exposure to these particles. Nanoplastics are particularly hazardous as they can penetrate biological barriers and absorb toxic compounds, which is facilitated by their small size and large surface-to-volume ratio. Traditional Fourier-transform infrared (FTIR) spectroscopy can only detect particles larger than $\sim 10 \mu\text{m}$, while Raman spectroscopy can identify particles down to 1 μm – and sometimes even 300–500 nm – but often suffers from fluorescence interference. This highlights the need for novel methods enabling adequate characterization of plastic particles in the sub- μm range.

Using Optical Photothermal Infrared (O-PTIR) spectroscopy, the accepted limitations of traditional IR microscopy are broken, providing for true submicron spatial resolution operating in contact-less reflection mode (as opposed to ATR), whilst providing for FTIR transmission/ATR-like data quality. Furthermore, it bridges the gap between conventional IR microspectroscopy and nanoscale IR spectroscopy. Now, for the first time, it also enables IR+Raman - simultaneous IR and Raman measurements at the same time, from the same spot and the same resolution. The complementary information gained by both IR and Raman scattering spectroscopy allows for cross-validation of results between different techniques. Additionally, the measurements can be combined with co-located widefield fluorescence microscopy, facilitating the identification of regions of interest and the localization of particles to be characterized. The measurements can be automated employing an algorithm called *featurefindIR®*, which enables fast evaluation of samples, both quantitatively and qualitatively.

Several applications of how O-PTIR can advance analytics of micro- and nanoplastics will be highlighted. O-PTIR has been applied in the characterization of particles extracted from water and consumer products like beer and yoghurt as well as embedded in various organic matrices.

3.18.P-Mo200 Identification of microplastics using Nile Red microscopy: case studies on wastewater and effluents from treatment plants

Audrey Joris, Aymeric Nagant and Laurence Haouche, ISSeP, Belgium

The determination of microplastics (MPs) in the environment is a growing concern. There is still no real consensus on the preparation and analysis methods to be used. Each laboratory applies the method it has developed. There are a lot of sources of error and misidentification in MPs analysis. This is due, on the one hand, to the complexity of this pollutant, its nature, its origins, its degradation in the environment and its affinity with other pollutants that adsorb onto these particles. On the other hand, there is a lack of comparability between the results obtained by different methods and different laboratories. This observation was made during interlaboratory studies as the Missouri project.

Identifying MPs using Nile red microscopy is an initial approach to observe MPs particles from 100 μm to 1 mm. It's a simple and inexpensive method for determining the presence of MPs in water. However, this method

has certain drawback. Observing the different areas of the filter is very time-consuming. This drawback can be reduced by using counting software.

The study concerned highlights the difficulties encountered when observing water samples and identifies the errors that can occur when applying this method. Despite the prior removal of organic matter (MO), MO remains a significant source of interference. MO causes either an overestimation because it fluoresces in the same way as MPs.

Observations confirms that this method has proven useful in the context of an exploratory study as an initial approach to determining the presence of microplastics in raw water and effluent from treatment plants. Analytical methods can provide more precise results, for example microFTIR or pyrolysis GC-MS. These techniques are more expensive, but provide more accurate data on the presence of MPs, including the type of plastic, the quantity and the concentration in the sample. More effective is the preparation sample phase, more accurate will be the MPs measure. There is a real need for CRM based on aged plastics in order to calibrate measurement methods more accurately.

3.18.P-Mo201 Development of a High-Resolution Analytical Method for Detecting Biodegradable Microplastics (>20 µm) in Surface Waters and Sediments Using Micro-FTIR Imaging

Yutaka Kameda, Chiba Institute of Technology, Japan

Production and commercial use of biodegradable plastics—including polylactic acid (PLA), polyhydroxybutyrate (PHB), polybutylene adipate terephthalate (PBAT), and polybutylene terephthalate (PBT)—have expanded rapidly as policy-driven substitutes for traditional polyolefins. In Japan, for example, agricultural films, seedling pods, and protective field materials are increasingly manufactured from these polymers, and governmental guidelines explicitly encourage their adoption to mitigate polyolefin-derived MP emissions to marine ecosystems. Biodegradable polymers are commonly assumed to mineralize into carbon dioxide and water via hydrolysis and microbial activity within soil or compost environments over timescales ranging from months to several years. However, emerging evidence suggests that their degradability in aquatic environments—including surface waters, sediments, and low-nutrient marine systems—may be substantially reduced. Several recent experimental and field studies indicate that these materials may persist for extended periods, and in some cases fragment into microplastic forms more rapidly than conventional polymers. This raises the possibility that biodegradable MPs may exhibit higher environmental mobility, broader dispersal potential, and distinct exposure-based ecological risks compared with traditional microplastics. Despite these concerns, virtually no systematic monitoring data exist regarding the environmental occurrence, distribution, or physicochemical characteristics of biodegradable polymer-derived MPs in aquatic systems. To address this critical gap, this study proposes and validates a high-resolution analytical workflow capable of detecting and identifying biodegradable MPs >20 µm in both surface water and sediment samples. The method integrates automated particle isolation with micro-Fourier transform infrared imaging, enabling polymer-specific mapping, size determination, and compositional classification of PLA, PHB, PBAT, and PBT particles with high reproducibility and spectral accuracy.

This presentation describes the development, optimization, and application of this analytical approach and discusses its potential for establishing baseline occurrence datasets and supporting future environmental risk assessments of biodegradable microplastics in freshwater and marine ecosystems.

3.18.P-Mo202 Biodegradability of Polymers in Aquatic Test Systems: ISO 14852 - Feasibility Study for Different Concentrations of Test Item and Inoculum

Andrea Brunswik-Titze, Jessica Buehler and Marie Canon, Hydrotex GmbH, Germany

Polymers are a product group that is increasingly becoming the focus in the topic of biodegradability and persistency. Due to their broad spectrum of properties, both synthetic and natural polymers play essential roles in everyday life. Guideline EN ISO 14852: Determination of the complete aerobic biodegradability of plastic materials in an aqueous medium — Method using analysis of the released carbon dioxides (July 2021) is one out of several to proof biodegradability of polymers in aquatic test systems. According to the Official Journal of the European Union, Entry 78, the EN ISO 14852 is one of the permitted test methods, to demonstrate that the polymer is degradable (group 4, three compartments).

The ISO 14852 guideline follows the OECD 301B guideline very closely in terms of testing principles, but the permitted concentration range of the test concentration and inoculum concentration is much larger (test concentration: 30 - 2000 mg L⁻¹ TOC (OECD 301B: 10 - 20 mg L⁻¹ TOC), inoculum concentration: 30 - 1000 mg L⁻¹ dry solids (OECD 301B: 30 mg L⁻¹ dry solids). Furthermore, according to the Official Journal of the EU, the test duration may be extended up to six months.

According to a ring test mentioned in the ISO Guideline, e.g. CO₂ evolution has only been determined in the

lower inoculum concentration range of 30 mg L⁻¹ dry solids. The study results presented here are intended to show the extent to which the range of permitted concentration levels (test item and inoculum) and test duration can be exploited while still complying with the validity criteria like CO₂ evolution in the blank controls or variation between the replicates. The results are from initial tests carried out with a water-soluble polymer, testing three different test item concentrations and three different inoculum concentrations with prolonged test duration. The resulting effect on the validity parameters required by the Guideline EN ISO 148521, but in particular on the biodegradability behaviour of the polymer, are presented.

3.18.P-Mo203 Fate of microplastic carbon: UV-radiation enhanced biodegradation of polyethylene and polystyrene traced with ¹³C isotopes in freshwater and Baltic Sea water

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Plastic pollution is perhaps among the most visible markers of human-driven environmental change in the Anthropocene. While plastic persists in the environment, it also fragments into micro- and nanoplastics that can eventually undergo microbial biodegradation to CO₂ and biomass. This disintegration depends on environmental factors like UV light and microbial communities. While these factors are recognized, their relative contributions to plastic degradation remain poorly quantified, especially in less-studied environments like freshwater and brackish waters. We used ¹³C-labelled polyethylene (PE) and polystyrene (PS) microplastics along with ¹³C-labelled starch as a positive control, to investigate plastic biodegradation and carbon pathways in humic lake water and Baltic Sea water over two months. Prior to incubation, labeled microplastics were irradiated in a solar simulator, with UV-A and UV-B daylight spectrum, corresponding to one summer of natural solar radiation in the Northern Hemisphere. Stable-isotope analyses of CO₂, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and microbial phospholipid fatty acids (PLFA) enabled precise quantification of plastic biodegradation and tracing carbon pathways. Additionally, we conducted microbiome analysis with 16S rRNA and analyzed microbial enzyme activity. UV exposure had a significant and systematic effect on plastic biodegradation in both waters, with largest response observed for PS in lake water. A notable fraction of labeled carbon resulted in DOC, indicating leaching before microbial assimilation. DOC from both plastic and starch was higher in Baltic Sea water than in lake water, possibly suggesting less efficient microbial uptake and processing in the brackish environment. Overall, both UV-light and water type affected plastic biodegradation rate, but UV-light had a more profound effect.

3.18.P-Mo204 Effects of Climate zones on plastic film degradation – Arctic to Tropic

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Plastics are recognized as an environmental threat and have been detected all over the planet. Plastics in the environment will degrade and fragment due to physical, chemical and/or biological forces, creating smaller-sized particles, including micro- and nanoplastics. Information regarding how plastic degrades and fragments in the environment is lacking.

Polyethylene (PE), Polypropylene (PP), and Polylactic acid (PLA) plastic film were produced from pellets without adding additives. The films with a thickness of 40–50 µm were exposed to summer outdoor conditions for 46 days in different climate zones, including Arctic, Tropic, Nordic, and Mediterranean zones. After exposure, the films were analyzed using Fourier Transform Infrared Spectroscopy (FTIR), tensile strength, and imaged using Scanning Electron Microscopy (SEM).

Preliminary results indicate the highest degradation for PP followed by PE, and there is no sign of degradation for PLA in the tested conditions. Moreover, we see a relation between temperature, sun intensity and the level of degradation.

3.18.P-Mo205 Environmental Aging and Cellular Interactions of Biodegradable Microplastics from PLA and PBS

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Biodegradable polymers such as polylactic acid (PLA) and polybutylene succinate (PBS) are increasingly used as alternatives to conventional plastics. However, like other polymers, they may fragment into biodegradable microplastics (BioMP) during the degradation process when exposed to environmental conditions. Their aging behavior and potential interactions with living cells remain insufficiently understood.

This study investigated how environmental aging influences the structural, thermal, and chemical properties of BioMP generated from PLA and PBS, and how aged particles interact with human fibroblasts in vitro. PLA and PBS pellets were ground into ~1 mm particles, and the grinding process increased their crystallinity. The crystallized BioMP were subjected to accelerated aging combining UV exposure (855 MJ/m²), a black standard temperature of 60 °C, and controlled humidity. Particle properties were analyzed using differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM).

To evaluate biological interactions, aged BioMP were tested in WI-38 fibroblasts proliferation and viability assay. This model enabled assessment of cell responses to particles that had undergone different aging conditions. Under the tested conditions, aging did not lead to detectable changes in the thermal transitions, chemical structure, or morphology of PLA and PBS BioMP. Preliminary in vitro results with WI-38 fibroblasts similarly indicated that aging did not influence the cellular response, as cells proliferated equally in the presence of both initial and aged samples over the 7-day experiment. These findings describe the behavior of PLA- and PBS-derived BioMP under specific laboratory conditions and provide a basis for future studies exploring broader environmental scenarios, extended aging periods, additional biological models, as well as the potential influence of additives and supramolecular structure on BioMP formation, aging, and biological interactions.

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3.18.P-Mo206 Aged Biobased Microplastics Release Toxic Compounds that Impair Early Algal Development

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Biobased plastics are increasingly adopted as alternatives to petroleum-based polymers due to their perceived environmental benefits. However, their degradation in marine environments may generate unknown transformation products with potential ecotoxicological relevance. This study examines how photochemical aging affects the toxicity of biobased microplastics and their released degradation compounds toward early developmental stages of *Ulva mutabilis*, a representative of the widely distributed *Ulva* genus that contributes to coastal primary production and nutrient cycling.

Microplastic particles of polylactic acid, polyhydroxybutyrate and cellulose acetate (1.125 mm) were aged under simulated sunlight in artificial seawater, and both particles and leaching extracts were tested in standardized algal bioassays. Toxicity was assessed using survival rate and chlorophyll a fluorescence of developing gametophytes. To further explore potential degradation-derived compounds, leaching extracts of aged cellulose acetate were analyzed using high-resolution mass spectrometry.

All polymers exhibited intrinsic toxicity, which substantially increased following aging. Photodegradation reduced median effect concentrations by factors of 4 to more than 10, with aged cellulose acetate producing the most toxic responses at < 1 mg/mL levels. High-resolution mass spectrometry revealed that aged cellulose acetate released a markedly broader suite of dissolved organic compounds than its nonaged counterpart, including numerous low molecular weight substances absent prior to aging. The emergence of these newly formed degradation products coincided with strong toxicity responses, indicating that transformation products, rather than solely pre-existing compounds, play a key role in the observed effects. Furthermore, toxic substances continued to leach after photodegradation had ceased, demonstrating persistent release beyond the exposure window.

These results demonstrate that biobased polymers can pose polymer-specific hazards after UV aging and challenge the assumption that biodegradability equates to ecological safety. The findings highlight the need to incorporate aging-related transformation products into environmental risk assessment of bioplastics and suggest

that regulatory evaluation should consider both inherent toxicity and degradation-dependent leaching in marine environments.

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3.18.P-Mo207 Does Environmental Weathering Alter the TED-GC/MS Fingerprints of Microplastics?
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Microplastic (MP) particles present in the environment exhibit considerable variability in size, morphology, and chemical composition due to the processes of aging and weathering. This variability complicates the analytical detection and identification of these particles, posing challenges for researchers and environmental monitoring efforts. Aging processes such as UV exposure, oxidation, mechanical abrasion and biological activity modify the physical and chemical properties of polymers, so that environmental MPs differ markedly from pristine reference materials. While thermoanalytical techniques have been used to study polymer aging, most work has focused on idealised systems and does not capture the complexity of real environmental samples. In this study, we apply thermal extraction–desorption gas chromatography–mass spectrometry (TED-GC/MS) in combination with laser-direct infrared (LDIR) imaging to pristine and environmentally weathered polymers (PS, PE, PET, PA-6,6, ABS, PVC, PP, CR) in order to (i) quantify the impact of weathering on thermal degradation kinetics and fingerprint intensity and (ii) assess how these changes affect the robustness of fingerprint-based polymer identification in complex matrices. Polymers were exposed to natural outdoor conditions using four different weathering procedures, including deployment on a rooftop and in a freshwater pond, with sampling after 3 and 6 months. Pristine and weathered polymer samples were subsequently characterised by TED-GC/MS and LDIR, and the resulting datasets were compared. The approach allows us to link changes in thermal degradation behaviour to alterations in chemical fingerprints and to evaluate to what extent environmental aging compromises, or preserves, the diagnostic value of TED-GC/MS for MP identification.

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The Chemical Water Quality laboratory at the KWR Water Research Institute was utilized for the execution of these experiments, for which we express our gratitude.

3.18.P-Mo208 Mapping the European Microplastic Landscape: Regional Emission Signatures, Pathway Effects, and the Mass-Number Discrepancy
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Mitigating microplastic pollution requires comprehensive, source-specific baselines that capture the distinct structural drivers across different geographies. This study presents a harmonized emission inventory for the EU-27, quantifying releases from seven major sources, spanning transport (tyres, road markings), industry (pellets), and consumer products (textiles, paints, scrubbing agents), using a systematic "Activity Level × Emission Intensity" framework. We estimate total annual emissions at 877 kt (536–1429 kt), corresponding to a per capita emission of 1.96 kg/year. On a mass basis, emissions are unequivocally dominated by tyre wear particles (87%) and pre-production pellets (7%), highlighting the critical footprint of the transport and industrial sectors. However, a paradigm shift occurs when emissions are assessed by particle count: textile microfibers emerge as the overwhelmingly dominant source (>99%), underscoring a fundamental disconnect between mass-based inventories and particle-driven ecological risks. Spatially, we identify distinct regional emission signatures, such as climate-induced road abrasion hotspots in Nordic countries and industrial pellet leakage in Western Europe. Furthermore, modeling of environmental pathways reveals that national wastewater infrastructure plays a critical role in mediating aquatic pollution; notably, Italy and France emerge as the largest contributors to surface water loads despite Germany being the largest generator. These findings demonstrate that a single metric is insufficient for risk assessment. We advocate for a dual-metric policy approach (mass and number) and regionally tailored interventions to effectively mitigate the diverse microplastic burden across Europe.

3.18.P-Mo209 Microplastics concentration in outdoor air: detection, quantification, identification

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Research on atmospheric microplastics (MP) remains limited compared to aquatic and soil studies. Early work relied mainly on particle-based spectroscopic polymer identification and passive sampling, reporting deposition rates of 29–1008 particles/m²/day, but long-term datasets are scarce. Compared to spectroscopic particle-based techniques, thermoanalytical methods offer faster quantification but with lower sensitivity.

This project aims to measure, quantify, and identify airborne MP and its sources using PM10 filter and deposition samples from rural background stations. Sampling is conducted every 2 weeks over two years at sites operated by the German Federal Environment Agency, representing coastal, lowland, forest, upland, and alpine environments. Deposition samples are collected at six stations using stainless-steel passive samplers, while PM10 samples are taken at three stations with small filter devices. Sampling began in November 2025, with first analyses scheduled for early 2026.

Sample preparation focuses on reducing matrix components that may interfere with detection. Deposition samples are sieved (<5 mm) and filtered (0.8 µm). If high amounts of pollen or organic debris are present, samples are gently dried and subjected to oxidative digestion. For PM10 samples, a key challenge is separating particles from the filter material; this is achieved through density separation using sodium iodide solution (1.8 g/cm³), allowing lighter MP particles to float and be isolated.

Analysis is carried out using thermo-extraction desorption gas chromatography–mass spectrometry (TED-GC-MS). This method enables identification of polymers, quantification of their mass fractions, and detection of tyre abrasion—which is difficult to assess with spectroscopic particle-based methods.

First results of these measurements will be presented along with the monitoring and sample preparation strategy.

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3.18.P-Mo210 Global Review of Microplastic Occurrence, Sources, and Methodological Gaps in Mountain Ecosystems

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Abundant even in remote mountain regions, yet their occurrence, sources, and impacts remain poorly understood. Mountainous ecosystems are highly vulnerable to climate-driven changes and anthropogenic pressures, making accurate monitoring essential. We conducted a systematic review of 70 peer-reviewed studies covering more than 900 sampling sites across global mountain systems to synthesise patterns of microplastic occurrence, sampling approaches, and methodological discrepancies. Water and sediment were the most studied compartments, while snow, ice, biota, and atmospheric fallout remain underrepresented. Reported microplastic abundances vary widely, often correlating with human accessibility rather than elevation alone. Analytical methods differ across matrices, including extraction techniques and polymer identification protocols, limiting comparability. Most studies report particle shapes and sizes, but fewer provide polymer composition or standardised units, complicating cross-study synthesis. Human Footprint Index (HFI) analysis revealed a strong bias toward pressured landscapes: ~75 % of studied sites were urbanised (HFI > 7), ~13 % moderate (4–7), and only ~9 % remote (HFI < 3), indicating that >90 % of investigations occurred near anthropogenically impacted areas. Tourism and atmospheric deposition were cited mostly as dominant sources at high altitudes. Our findings highlight critical gaps: lack of studies in several continents, non-harmonised methodologies and limited multi-compartment sampling. Addressing these gaps through standardised protocols and integrated monitoring is essential for assessing cumulative risks and informing conservation strategies for mountain ecosystems, which provide vital biodiversity and water resources for human health.

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3.18.P-Mo211 Mitigating Microplastic Loads in Wastewater Treatment Plants Serving Alpine streams

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Alpine streams, often considered pristine, are increasingly impacted by climate change, land-use alterations, and emerging pollutants such as microplastics (plastic particles smaller than 5 mm). These particles originate mainly from wastewater effluents, urban runoff, and agricultural activities, posing ecological risks to freshwater ecosystems. However, monitoring microplastics remains difficult due to variations in sampling methods and reporting units, which limit comparability across studies. To improve monitoring and evaluate mitigation strategies, we tested a novel filtration system in three wastewater treatment plants (WWTPs) in South Tyrol, Italy, using a Before-After-Control-Impact design. We combined a rapid habitat sampling method with a microplastic discharge unit (MDU) calculation to assess changes in microplastic abundance in river water, sediment, and macroinvertebrates upstream and downstream of WWTPs before, during, and after filter installation. Microplastics were detected in all compartments, with fibres as the dominant type. Preliminary results indicate spatial differences in concentrations and suggest that discharge variability influences reported abundances. The filtration system demonstrates potential for mitigating microplastic loads, supporting compliance with the new EU Urban Wastewater Treatment Directive. These findings contribute to improving monitoring approaches and inform strategies to protect Alpine freshwater ecosystems, which are biodiversity hotspots and providers of essential ecosystem services.

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3.18.P-Mo212 Quantifying Loads and Removal Efficiencies of Elastomeric Particles, Including Tire- and Road-Wear Particles, in Two Municipal Wastewater Treatment Plants in Japan

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Understanding the environmental behavior and pathways of tire and road wear particles (TRWPs) in urban systems is essential for mitigating their discharge to aquatic environments. Previous studies indicate that urban road runoff can contain high concentrations of TRWPs, particularly during rainfall events. In cities with combined sewer networks, such runoff is transported to wastewater treatment plants (WWTPs), whereas in separate systems it may bypass treatment and enter receiving waters directly. In addition, other elastomer-derived particles, including degradation products of 6-N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), are generated during the use and abrasion of rubber products. These fine particles can enter stormwater, atmospheric dust, and municipal wastewater even under dry conditions, suggesting continuous input to WWTPs irrespective of rainfall. Despite increasing concern, quantitative data on the loads, fate, and removal efficiencies of elastomer particles and TRWPs in WWTPs remain scarce. In particular, time-resolved measurements are lacking, hindering accurate estimation of emission factors and environmental transfer pathways.

In this study, influent and effluent waters from two WWTPs—one operating a combined sewer system and the other a separated system—were sampled hourly during dry weather using automated samplers. Fine particles (>20 µm) belonging to nine elastomer polymer classes, including TRWPs, were identified and quantified using a micro-Fourier transform infrared spectroscopy method developed for this study. Concentrations in sewage sludge were also measured to characterize partitioning behavior and assess retention during treatment. The resulting datasets provide new insights into temporal variability, influent loading trends, and polymer-specific removal efficiencies of elastomeric particles in contrasting wastewater treatment configurations. These findings improve understanding of the role of WWTPs in the urban lifecycle of rubber-derived particulate pollution and contribute essential parameters for predicting their fate in aquatic environments.

3.18.P-Mo213 Binding Properties of Heavy Metals on Tire and Road Wear Particles in the Aquatic Environment

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Tire wear particles (TWP) are an emerging environmental issue associated with growing ecotoxicological concern. In response the European Parliament has introduced the Euro 7 regulation, which will establish limits for tire abrasion. Simulations estimate that more than 100,000 tons of tire wear particles are released in Germany every year, representing roughly one third of all microplastics entering the environment. These

emissions are expected to rise due to increasing vehicle numbers.

Tire and road wear particles (TRWP), formed by friction between tires and the road surface, consist of elongated rubber particles (10–350 µm) that incorporate additional particles from the road. TRWP contain Zn (from the manufacturing process) as well as heavy metals like Cr, Ni, Cu, Cd and Pb, which originate mainly from road traffic. Rainfall mobilizes these composite particles and transport them into the aquatic environment via gullies and drainage systems, where TRWP may release incorporated heavy metals into the surrounding water, potentially making them bioavailable to aquatic organisms.

To assess the risk of heavy metal release from TRWP and their potential bioavailability, we applied a sequential extraction procedure (Community Bureau of Certified References, BCR) to TRWP samples collected from a highway tunnel and go-kart tracks. This extraction method includes three steps simulating different aquatic conditions: slightly acidic (readily available elements, aerobic, F1), reducing (metal oxides, anaerobic, F2), and oxidising (bound to organic matter, F3) and a residual fraction (F4).

TRWP from the Elbe tunnel exhibited high bioavailability of Cd and Zn (> 50% in F1), while Pb was predominantly mobilized under reducing conditions (> 70% in F2). The investigated trace elements followed the mobility ranking: Zn > Pb > Cd >> Cu > Co > As >> Ni > Cr. Furthermore, incubation of TRWP with river water from the Freiburger Mulde (high heavy metal concentrations) revealed that newly adsorbed Cd and Zn to TRWP from the surrounding water were only weakly bound and easily bioavailable (> 95% Cd, > 85% Zn). These findings indicate that TRWP can significantly influence the mobility and bioavailability of heavy metals in aquatic systems. This has important implications for evaluating ecological risks and for understanding the role of TRWP as vectors transporting toxic metals to aquatic biota.

3.18.P-Mo214 Microplastics Monitoring in the Dutch Rivers - Small Temporal and Spatial Variations in Microplastic Concentrations in Suspended Matter

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Over the past 5 years, Rijkswaterstaat developed and validated sampling and analysis methods for the monitoring of microplastics in the Dutch rivers. To sample suspended particulate matter (SPM), including microplastics, from rivers in a representative way, the performance of two sampling methods, both suited to sample large water volumes, was evaluated. First, time-integrated samples were taken by continuously pumping water through a sedimentation box for periods of 2 to 4 weeks. The performance of the method was evaluated by sampling with 2-4 set-ups at the same location during the same period, showing good reproducibility and reproducible retention of SPM. In addition, the influence of sampling depth was studied by sampling at different depths (10-290 cm). The second sampling method studied was SPM sampling using a continuous flow centrifuge. With this discrete sampling method, large water volumes can be sampled in short periods of time (hrs). The composition (particle size and microplastic composition) of SPM collected by both methods were compared and showed only small variation. Particle size distributions of the SPM sampled by sedimentation box or flow centrifuge, from different sampling locations and different sampling periods were similar: over 90% of the sampled SPM was smaller than 75 µm. Large, visible microplastics were rarely found.

Microplastic concentrations in samples collected at multiple locations along the Dutch sections of the Meuse, Rhine and Scheldt were measured using thermal extraction desorption gas chromatography – mass spectrometry, using a validated method with a reproducibility below 20%. In all samples, polyethylene and styrene-butadiene rubber were the most abundant polymers. Polypropylene, polystyrene and natural rubber were also detected at concentrations above the limit of quantification in most samples. Microplastic concentrations in SPM sampled by time-continuous sampling with the sedimentation box or discrete sampling with the flow centrifuge showed only small variations. While SPM concentrations (mg/L) show large variations in time and space, microplastic concentrations within the SPM (g/kg) show only minor temporal and spatial variations. No correlation between microplastic concentration and river discharge was found. These findings suggests that SPM based monitoring could provide a reliable approach to assess microplastic pollution in riverine environments.

3.18.P-Mo215 Polymer-Specific Characterization of Microplastics (>20 µm) in Surface Waters of the Klang River, Malaysia, and Their Estimated Flux to the Coastal Ocean

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Rivers are now recognized as major pathways transporting land-based microplastics (MPs) to marine ecosystems, yet quantitative field measurements describing their fluxes—particularly from large tropical rivers in rapidly developing regions—remain limited. Southeast Asia, where plastic consumption, population density,

and watershed connectivity converge, represents a critical but undercharacterized source region. To address this gap, we conducted a systematic assessment of the abundance, polymer composition, and size distribution of MPs in the surface waters of the Klang River, Malaysia, and developed a first-order estimate of their export flux to the adjacent coastal sea.

Surface water samples were collected at 19 sites along a ~90 km transect spanning the system from the upper reaches of the Batu River, through the midstream Klang River corridor, to the tidal estuary at Port Klang. Sampling was conducted during the local dry season (June 2025) to capture low-flow transport conditions. At each site, 10–50 L of surface water were filtered in situ using a 10 µm plankton net, and river discharge was quantified via the velocity–area method based on cross-sectional geometry and float-derived surface flow velocity.

In the laboratory, retained material was rinsed from the net, subjected to oxidative digestion using hydroperoxide solution, and subsequently processed by density separation in sodium iodide. The supernatant was recovered in triplicate, combined, and filtered onto silicon membranes. Particles larger than 20 µm were analyzed using micro-Fourier transform infrared (micro-FTIR) imaging spectroscopy to identify 16 polymer types, enabling polymer-specific quantification.

Near the river mouth during low tide, surface water contained 38 MP particles L⁻¹. When scaled using discharge measurements, this concentration corresponds to an estimated export flux of 2.3×10^7 particles s⁻¹, equivalent to approximately 30 g s⁻¹ of plastic transported toward the coastal ocean. These results demonstrate that a single urban river system can deliver tens of millions of microplastic particles per second under typical hydrological conditions, highlighting the significance of riverine pathways in sustaining marine plastic pollution. The findings underscore the urgency of improving watershed-scale waste management and monitoring frameworks in rapidly urbanizing tropical regions.

3.18.P-Mo216 SPATIOTEMPORAL PATTERNS IN MICROPLASTIC MORPHOLOGY AND COLOR IN THE PARAÍBA DO SUL RIVER AND THEIR ECOLOGICAL IMPLICATIONS

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Microplastic ingestion has been documented across various trophic levels. Mechanisms include misidentification of food and trophic transfer. Physical characteristics such as size, shape, color, and buoyancy significantly influence the likelihood of interaction. The present study characterizes the morphology and color of microplastics (MPs) in the Paraíba do Sul River during sampling campaigns in May and September 2025. The study focuses on spatiotemporal variation and the potential ingestion risk to aquatic biota.

Microscopic analysis identified particles ranging from 50 to 500 µm, including fragments, fibers, films, foams, and granules. In May, fragments constituted 66% of the total, while fibers accounted for 22.7%. Transparent, black, and blue particles were most frequently observed. Sampling points 8, 9, and 10 exhibited the highest particle counts (150, 121, and 115, respectively). Notably, downstream locations displayed higher concentrations but reduced color diversity. In comparison, the September sampling revealed a substantial increase in the total amount of microplastics compared with May. This rise can be attributed to periods of intense rainfall, which elevate microplastic loads in water bodies by mobilizing accumulated urban waste. Consequently, between the first and second sampling campaigns, the total number of microplastics increased markedly across all sites, rising from 26–150 particles per point to 87–230 particles per point. There was substantial growth in all morphological categories, with pronounced increases in transparent (+75.2%) and black (+101%) particles, and the emergence of orange particles, which were absent in the first campaign. These observed spatiotemporal changes suggest hydrodynamic, anthropogenic, and seasonal influences. Particles in this size range are ingested by zooplankton, mollusks, aquatic insect larvae, and small fish. This may interfere with feeding, promote satiety, disrupt digestion, and hinder growth. Higher concentrations downstream show that these areas accumulate pollutants. Different polymers show distinct ingestion and retention patterns. Biomagnification speeds contaminant transfer and increases ecological risks at higher trophic levels.

3.18.P-Mo217 Plastic pollution in urban freshwater systems: characterization in two contrasting lagoons of Buenos Aires, Argentina.

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Plastic pollution in freshwater systems is an emerging environmental issue, particularly in densely populated urban areas. Urban lakes and lagoons in Argentina have been shown to contain microplastics, but integrated

assessments of macro- and microplastics, their weathering state, and associated risks remain scarce. This study characterizes plastic pollution in two contrasting urban lagoons in Buenos Aires Province: one highly urbanized lagoon in the Buenos Aires Metropolitan Area (AMBA) (high anthropogenic pressure, HU) and one moderately urbanized lagoon in Tandil (lower anthropogenic pressure, LU). The objectives were to: (i) monitor the presence and polymer composition of macro- and microplastics; (ii) quantify the degree of surface weathering of collected plastics; (iii) assess the ecological risk of these lagoons in relation to plastic pollution. Macro- and microplastics were collected from the water column and littoral zones, classified by morphology and polymer type, and analysed using Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR) to calculate carbonyl (CI), hydroxyl (HI), and silicon (SI) indices as proxies for environmental weathering. An ecological risk index for microplastics (RI) was estimated for each lagoon to quantify plastic-related ecological threats.

The HU lagoon showed higher contamination, with microplastic abundances of 9 ± 4 items/L and macroplastics of 0.6 ± 0.3 items/m², compared with 1.4 ± 0.7 items/L and 0.1 ± 0.1 items/m² in the LU lagoon. Polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) were the most abundant macroplastic polymers, with PE exceeding 35% in both lagoons. PP was the predominant polymer among microplastics in both lagoons. PE macroplastics from HU exhibited significantly higher weathering, reflected in elevated CI, HI, and SI values, whereas no consistent differences were detected for PP or PET. The average ecological risk index for microplastics was 38.7 ± 23.4 (low to high risk) in LU and 134.0 ± 76.8 (high to extremely high risk) in HU, highlighting the influence of urbanization on both plastic loads and associated risks.

These findings show that urban intensity shapes the quantity, composition, and degradation state of plastic debris in small freshwater systems and provide a risk-based framework to inform monitoring, mitigation, and management of plastic pollution in urban water bodies across South America.

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3.18.P-Mo218 How Do Microplastics Distribute Through Freshwater Ecosystems? The Search for a Bioindicator Species

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Microplastics (MPs) have been detected in the freshwater ecosystems globally. Microplastics are tiny synthetic materials usually between 5 mm to 0.01 mm. Although research on MPs in freshwater ecosystems lagged behind that in the marine environment, this gap has narrowed over the last decade. When MPs enter freshwater environments, they do not simply transport downstream; MPs' distribution is impacted by the naturally heterogeneous physical characteristics of rivers. Abiotic components, such as depth, discharge, velocity, and sediment grain size, and biotic components, such as vegetation and biota, can all affect how MPs distribute through a river system. This in turn changes the MP load available to biota and their ability to ingest higher quantities of these particles. Microplastics have also been detected in almost all major animal groups, including freshwater invertebrates, fish, reptiles, amphibians, birds, and mammals. Globally, fish have been among the most investigated groups, with over 100 species studied. This group was closely followed by invertebrates, then birds, and, lastly, reptiles, mammals, and amphibians, which were the least investigated groups. Concentrations found within biota varied greatly from 1 particle per animal to over 50 particles. The foundational information of animals that ingest MPs is key to determining whether a species can be considered a bioindicator for MPs in freshwater environments. Given the wide variety of methods for sampling MPs in rivers, a bioindicator species might bridge the gap created by differences in sampling and reporting methods. Currently, fish species such as *Cyprinus carpio* and *Oreochromis niloticus*, which are widely distributed, omnivorous, invasive, and easily identified, have been found to ingest MPs across multiple continents and could be good bioindicator species. Other animals, such as chironomid larvae, oligochaetes, freshwater mussels, clams, and other filter-feeding invertebrates, have also been suggested as bioindicator species. With MP research ready to turn to more monitoring-style research, a good bioindicator species could yield more representative and comparable data across study locations globally; however, more research is required. Final considerations, such as increased research on underrepresented groups, such as birds, amphibians, and reptiles, must be made to understand their interactions with MPs, which is still unknown.

3.18.P-Mo219 Assessment of Polymer-Specific Deposition Dynamics of Microplastics (>20 µm) Using Sediment Traps in a Eutrophic Lake

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The vertical transport and deposition dynamics of microplastics (MPs) represent a critical yet insufficiently resolved component of their environmental fate in aquatic ecosystems. Understanding when, how, and which polymers transition from the water column to benthic environments is essential for predicting long-term accumulation, ecological exposure, and biogeochemical implications. Although most synthetic polymers—particularly polyolefins—exhibit densities comparable to or lower than freshwater and seawater, numerous studies now suggest that aggregation processes can facilitate particle settling. Interactions with suspended inorganic minerals, microbial biofilms, phytoplankton, and extracellular polymeric substances can modify the physicochemical properties of MPs, effectively increasing particle density, hydrodynamic drag, and settling efficiency. However, key knowledge gaps remain regarding the polymer-specific characteristics of MP aggregates—including their size distributions, composite densities, and morphological features—that govern deposition rates and fluxes across diverse aquatic settings. Despite growing concern, empirical field observations of MPs >20 µm settling in natural waters remain limited, particularly in eutrophic lake systems where elevated primary productivity and high organic matter concentrations may accelerate aggregation and deposition relative to marine systems. Eutrophic lakes therefore represent ideal natural laboratories to assess polymer-dependent sedimentation behavior under biologically enhanced particle collision environments. In this study, we present results from a field monitoring program designed to quantify the deposition behavior of MPs larger than 20 µm in one of Japan's most eutrophic freshwater lakes. Sediment traps were deployed at 1.0, 2.5, and 4.5 m depths and retrieved at two-month intervals to capture temporal variation in deposition fluxes. Retrieved particles were analyzed using micro-Fourier transform infrared imaging, enabling polymer-specific identification of 16 different materials. In addition to polymer classification, the workflow allowed measurement of size distributions of MP-containing aggregates and characterization of polymer-dependent deposition patterns.

3.18.P-Mo220 Polymer-Specific Density Distributions of Microplastic Aggregates Reveal Their Differential Settling Behaviors in Tokyo Bay Seawater

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Understanding the fate of microplastics (MPs) in marine environments remains challenging, particularly due to the limited availability of long-term, high-resolution observational datasets. A persistent discrepancy exists between global MP load estimates and concentrations reported by field monitoring programs. In particular, in situ quantification of settling fluxes and deposition velocities is technically difficult, as sediment-trap deployments require substantial cost, labor, and long-term maintenance. Consequently, many previous studies have relied on theoretical sedimentation models—most commonly Stokes-type equations—to infer MP depositional dynamics. However, these models require accurate measurements of particle size and effective density in situ, and such datasets are scarce, especially for MP-containing aggregates in coastal seawater. In this study, we present the first monitoring results characterizing both size distributions and in situ density spectra of MP-bearing aggregates (>20 µm) in the seawater of Tokyo Bay. Aggregates were collected using a 10-µm plankton net and subsequently fractionated into discrete density classes via cascade density separation using sodium iodide solution. Each density fraction was digested using a modified Fenton reaction, and MPs >20 µm were identified, quantified, and polymer-typed using imaging micro-Fourier transform infrared spectroscopy. Microscopic imaging prior to digestion enabled direct measurement of aggregate dimensions, providing pair-matched datasets of size, density, and polymer composition. Using these empirically derived values, polymer-specific deposition velocities were estimated based on theoretical settling models. The resulting deposition patterns were further compared with polymer compositions of MPs in seabed sediment samples from Tokyo Bay to evaluate consistency between predicted and observed depositional signatures. This integrated monitoring and analytical framework provides rare observational evidence for polymer-dependent settling behavior of MP-containing aggregates in a semi-enclosed coastal system and offers a methodological pathway to reduce uncertainty in global MP fate modeling.

3.18.P-Mo221 Half-century record of coastal microplastic fluxes (>20 µm) in a sediment core from Nanao Bay, Japan

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Microplastics (MPs, $\geq 20 \mu\text{m}$) preserved in dated sediment cores provide a means to reconstruct temporal trends in plastic contamination. We analyzed MPs in a gravity core collected from western Nanao Bay, a semi-enclosed coastal embayment on the Sea of Japan. The core was sliced at 1–2 cm resolution and dated using ^{210}Pb (CFCS model) with support from ^{137}Cs . After removal of organic matter and density separation, particles 20–5000 μm were collected on 5 μm silicon filters and identified by micro-FTIR imaging. For each slice, MP fluxes were calculated by multiplying dry-weight concentrations (items g^{-1}) by layer-specific dry mass accumulation rates ($\text{g cm}^{-2} \text{yr}^{-1}$). Across the 1960–2012 record, MP fluxes ranged from 4.2 to 122.4 items $\text{cm}^{-2} \text{yr}^{-1}$, which is within the range reported for microplastics in Great Lakes sediment cores.

Total MP fluxes in the Nanao Bay core increased from about 4.2 items $\text{cm}^{-2} \text{yr}^{-1}$ around 1960 to 45.8 items $\text{cm}^{-2} \text{yr}^{-1}$ around 1980, broadly paralleling the rapid growth of plastic production and use in Japan. In contrast, fluxes during 1990–2000 decreased to 11.3–16.1 items $\text{cm}^{-2} \text{yr}^{-1}$, similar to levels in the mid-1960s. This decline is consistent with changes in plastic consumption patterns in the catchment, the expansion of municipal wastewater treatment plants around 1990, improvements in solid-waste collection, and rising public concern about litter and waste management. Superimposed on these long-term trends, several core slices show fluxes approximately one order of magnitude higher than adjacent layers; these peaks coincide with years when major typhoons affected the Nanao Bay region, indicating short-term event-driven increases in MP delivery to the seabed.

Overall, the Nanao Bay record shows that temporal variability in MP fluxes to coastal sediments is broadly consistent with the historical trajectory of plastic production, and is also consistent with possible influences from patterns of use, wastewater and solid-waste management, public awareness, and episodic extreme weather events such as typhoons.

3.18.P-Mo222 Transformation Patterns of Environmental Plastic Pellets

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Since the 1970s the widespread presence of industrial plastic pellets have been observed in surface waters and beaches all over the world. In the port of Antwerp (Belgium), located in the Scheldt estuary, pellets are being unintentionally released whereafter they are exposed to a variety of environmental stressors (temperature, UV radiation, salinity, water, oxygen, biological activity, and mechanical stress) causing various patterns of physical and chemical degradation. Our aim is to identify transformation patterns of released pellets to better understand the mechanisms underlying their breakdown.

Plastic pellets were collected in the port area (road and roadside) and throughout the Scheldt estuary (riverbank, soil, water and sediment) between 2022 and 2025. Confocal microscopy ($n = 78$) and MultifileAnalyzer were used to examine the pellet's surface roughness. Micro-CT scanning ($n = 24$) and DragonFly World were used to examine the interior of the pellets.

Pristine pellets (polyethylene/polystyrene) from Antwerp producers were used as reference materials and showed clean-cut edges, smooth surfaces and texture lines. Four transformation patterns were identified: (1) accumulation of contaminants, (2) textural changes in terms of increased surface roughness or abrasion, (3) the formation of cracks and (4) the formation of internal holes. Environmental pellets showed higher surface roughness than pristine ones. Cylindrical pellets exhibited less uniform texture and less additional surface area compared to their pristine counterparts. Polystyrene pellets are more likely to release smaller particles due to texture abrasion, while polyethylene pellets lose smaller particles via external cracks.

Transformation processes start from the moment a pellet enters the environment. Material exchange occurs between the plastic and its environment: the pellet's chemical structure breaks down into micro- or nanoparticles, potentially releasing polymer additives, and environmental substances enter the pellet.

Degradation processes reinforce each other: increased surface roughness enhances the possibility of retaining contamination and the release of smaller plastic particles.

The next step in this research will be to determine the polymer types and the oxidation indices. This will allow us to better understand the polymer-specific breakdown mechanisms— both in terms of transformation patterns and chemical breakdown due to oxidation – in order to assess their potential risk.

3.18.P-Mo223 Polyolefin-based coated fertilizer fragments ($\geq 20 \mu\text{m}$) as microplastics in rivers and a coastal bay in Japan

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Polymer-coated fertilizers are increasingly used to reduce labour input and enhance nutrient-use efficiency through controlled nutrient release. In many formulations, fertilizer granules are encapsulated by a polyolefin-based shell that undergoes gradual degradation in soils and surface waters, allowing dissolved nutrients to diffuse outward over time. As the shell cracks and delaminates, coating fragments detach from the granules and can persist as microplastic particles. Coated fertilizers are therefore a plausible primary source of microplastics to agricultural soils and hydrologically connected waters. Yet, despite this concern, field observations that unambiguously identify microplastics derived from coated fertilizer coatings, particularly in the 20 µm size range, remain very scarce.

Chandra et al. (2019) estimated that coated fertilizers account for only about 0.25% of total fertilizer use globally and about 8–10% and 1% of fertilizer use in Europe and the United States, respectively. In Japan, an ageing agricultural workforce has driven demand for labour-saving fertilization practices, and the use of coated fertilizers is expected to increase accordingly. This trend heightens the need to understand the environmental fate of polyolefin coatings and their contribution to microplastic loads.

This study investigates the occurrence and abundance of polyolefin-based coated fertilizer fragments (20 µm) in riverine and coastal environments in Japan. Environmental samples were collected in September 2025 from four sites along the Kumaki River, one site along the Otsu River and one site in Nanao West Bay. For all six sites, the presence and abundance of polyolefin-based coated fertilizer particles >20 µm were confirmed and quantitatively assessed.

By providing site-resolved data on coating-derived microplastics in both rivers and a semi-enclosed coastal bay, this work identifies polyolefin-based coated fertilizers as a diffuse agricultural source of microplastics that directly links fertilization practices to microplastic contamination. These findings support the inclusion of coated fertilizer use and management in future source apportionment and risk assessments of microplastics in intensively farmed regions.

3.18.P-Mo224 Microplastic Formation from Biodegradable and Conventional Mulching Films: Soil Degradation Study

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Agricultural mulching films contribute significantly to microplastic contamination in soils. In this study, the degradation and release of microplastics from three mulching films were investigated over 180 days in a soil matrix under environmentally relevant conditions. Tested materials included two conventional LDPE films (black and transparent), one biodegradable PBAT/starch film. Pristine and UV-weathered forms of each film were included. Additionally, a field-weathered black LDPE film.

Degradation of plastic fragments > 2 mm was quantified via weight loss, while the soil fraction < 2 mm was collected for microplastic analysis. A method for extracting microplastics from soil was optimized by combining oleo-extraction with an additional density separation step. Extracted particles were characterized using FTIR and LDIR chemical imaging to determine their polymer composition, particle count, and particle size distribution.

After 180 days, biodegradable films showed the highest degradation (up to 15 % weight loss), whereas conventional films exhibited minimal fragmentation (< 5 %). UV pre-treatment slightly increased the fragmentation of biodegradable films. Microplastic analysis revealed higher particle counts from biodegradable mulching films compared to conventional mulching films.

These findings demonstrate that biodegradable mulching films degrade more rapidly than conventional mulching films, producing measurable microplastics under realistic soil conditions. Optimized extraction and characterization methods provide robust tools for quantifying microplastic generation and evaluating the environmental implications of biodegradable agricultural plastics.

3.18.P-Mo225 Challenges to determine polymers in soil by Py-GC/MS

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Plastics in the environment are a major global challenge of the 21st century. Large amounts enter the soil in various ways. Relevant entry paths include tire abrasion, sewage sludge, mulch films, and compost. Sample preparation and analysis of plastics, especially in the <1 mm fraction (SMPs, small microplastics), is subject to various analytical and methodological challenges.

The project “SOSMA” (soil sampling for microplastic analysis) investigated the entry of plastics into an arable soil (Cambisol) via the compost pathway. A total of 173 samples were analyzed for macroplastics (MaPs, >5 mm) and large microplastics (LMPs, 5-1 mm). A subset was examined for SMPs (<1 mm). First, the focus was on sample preparation and the determination of recovery rates using pyrolysis gas chromatography coupled with mass spectrometry (Py-GC/MS). The samples (master sample = 2 L, tested subsample 500 g) were treated with density separation and Fenton digestion after dry sieving. Cryogenic grinding was also performed for homogenization as the particulate plastics were inhomogeneously distributed in the samples after processing. The recovery rates of spiked plastics (polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS)) were determined using Py-GC/MS from the solids and after solvent extraction. Second, both methods were applied to the unspiked soil samples.

Further homogenization by cryo-milling reduced the inhomogeneity for PP and PS. However, grinding may lead to a larger mineral surface area, which can result in lower recovery rates due to changed pyrolysis conditions (PE). The recovery of PET was at a low level, which is why no data interpretation is provided for PET. The recovery rates of the common three (PE, PP, PP; 0% - 225%) show partly acceptable but method and polymer specific results. Hence, no combination of methods (unmilled/ cryomilled; solid/ solvent extraction) provides the perfect result to determine all investigated polymers.

The results for the unspiked soil samples show higher polymer concentrations after solid matter measurement compared to the measurement after solvent extraction. More results regarding the unspiked samples will be available at the time of the conference. However, for a representative and reliable quantification of plastics in soils, it is necessary to better understand the polymer specific causes of sample preparation and matrix effects on the analysis results derived by Py-GC/MS.

3.18.P-Mo226 Down Profile Migration of Microplastics in Agricultural Soils Amended with Composted Municipal Solid Waste: Influence of Soil Type, Application Method and Temporal Dynamics

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Applying composted municipal solid waste (MSW) to agricultural soils can enhance soil health and crop productivity. However, the presence of microplastics (MPs) has raised concerns about potential adverse effects on soil systems and food security. Despite these concerns, the migration of MPs in different soil types, under different compost application methods and over temporal scales remains poorly documented. Our study focused on the vertical distribution of MPs derived from composted MSW applied to three soil types: silty loam, sandy loam, and clay. A controlled glasshouse column experiment was conducted using soil columns (0.85 m length × 0.15 m internal diameter). Composted MSW was applied at 50 t/ha using two methods: surface application and incorporation to 15 cm depth. Columns were watered to maintain 60% field capacity. Soil samples were collected at 5 cm depth intervals up to 50 cm at 12 and 18 months. The MPs were extracted using H₂O₂ (30%) digestion and ZnCl₂ density separation, then quantified and characterised using stereoscopic zoom microscopy. Surface application of composted MSW resulted in high retention (>44%) of MPs within the 0–5 cm layer across all three soils at both time points. In the incorporated treatment, MPs were largely retained within the top 15 cm (30–40% in 0–5 cm and 5–35% in 5–10 cm). Higher MP counts were observed in the sandy loam compared to similar depths for other soils, but this was influenced by the higher MP recovery from this soil. Below 20 cm depth, a higher number of MPs (3–49) were detected in incorporated compared with surface applied (1–17) across all soil types at 12 and 18 months. Surface application resulted in higher MP accumulation in the 0–5 cm layer, whereas incorporated MSW showed greater extent of vertical MP migration for all three soils. A small fraction of MPs was detected at 50 cm depth in all soil types under both application methods at the two sampling times. Morphological analysis showed that MP fibres were the most common, comprising 52–60% in surface treatments and 36–48% in incorporated. Size fraction analysis indicated that particles ranging 0.1–1 mm were most abundant, representing 44–55% of MPs across all treatments and depths. This study demonstrates that MPs in soil amended with MSW mobilise down profile and raises concerns regarding soil health, groundwater contamination, and off-site environmental impacts of MPs when utilising composted waste materials in agricultural applications.

3.18.P-Mo227 Earthworm-Facilitated Transport of Microplastics in Soil: A Small-Scale Field Lysimeter Study

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The application of plastic mulching films in agriculture is increasing, leaving persistent microplastic particles in soil where they are difficult to remove. Earthworms can mix soil and litter and may transport microplastics vertically, but evidence from realistic field conditions is still limited and sometimes inconsistent. We established a small-scale field experiment in Podgorica, Montenegro, to investigate how local earthworms affect the vertical transport of microplastics originating from aged mulching films. Cylindrical PVC tubes were used to construct small-scale lysimeters (110 mm inner diameter, 250 mm height), installed flush with the soil surface and connected to a smaller lower tube that served as a passive leachate reservoir, separated by a metal mesh and a layer of gravel. The upper part was filled with local field soil and covered with turfgrass collected on site. Aged mulching film was cut and shredded into four size fractions and applied on the soil surface (0.3 grams per lysimeter) under the turfgrass. Two treatments were used: with and without local earthworms, each with three replicates. The experiment ran for three months under semi-passive conditions, relying on natural rainfall supplemented by occasional irrigation events to support earthworm activity. At the end of the exposure, lysimeters were retrieved. Soil columns were sectioned into depth intervals, leachate was collected from the reservoirs, and all earthworms were recovered where possible. Microplastics were extracted using density separation with saturated salt solution and quantified by stereomicroscopy; leachate samples were filtered and examined for the presence of particles. We hypothesize that earthworm activity will enhance downward transport of mulching film microplastics compared with controls, but that the overall penetration depth will remain modest under field conditions relative to laboratory soil column studies. We expect most particles to remain in the upper soil layers, with limited detection in leachate and a stronger downward shift in smaller size fractions. Final quantitative data on vertical microplastic profiles, leachate, and earthworm responses will help clarify how microplastics are likely to move in agricultural soils and whether they represent a realistic pathway towards deeper soil layers and groundwater.

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3.18.P-Mo228 Comparative ecotoxicological assessment of microplastics across multiple species from marine and freshwater environments

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Humans have introduced numerous contaminants into ecosystems, including microplastics (MPs), raising major concerns regarding the health of natural ecosystems and organisms at various biological levels. This study assessed the potential effects and environmental hazard of polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and high-density polyethylene (HDPE) on six freshwater and marine model species: the microalgae *Raphidocelis subcapitata* and *Tetraselmis chuii*, the crustaceans *Daphnia magna* and *Artemia salina*, the bivalve *Mytilus galloprovincialis* and the fish *Danio rerio*.

All four MPs were tested on the six species at the same concentration range, 1 to 100 mg/L. The microalgae *T. chuii* and *R. subcapitata* were exposed to 72 h (ISO 10253; OECD 201, respectively). Immobilization of *A. salina* and *D. magna* was assessed after 48 h (ASTM E1440-91 adapted; OECD 202, respectively). In *M. galloprovincialis*, mortality and oxidative stress and neurotoxicity-related biomarkers were evaluated following the ASTM E729-96 (adapted). Finally, the *D. rerio* embryo toxicity test (OECD 236) aimed to record acute effects, malformations, and hatching delay over 96 h, and biochemical effects on larvae exposed until 120 h. No significant growth inhibition effects were detected in any microalgae species (*T. chuii* and *R. subcapitata*), nor immobilization on crustaceans (*A. salina* and *D. magna*), nor mortality on mussels *M. galloprovincialis* and *D. rerio* fish larvae (EC50 values > 100 mg/L). Nevertheless, significant biochemical responses indicated that, despite the absence of individual-level toxicity, subcellular effects were triggered.

Regarding biochemical effects on *D. rerio* larvae, PVC induced the most significant responses, increasing catalase, glutathione peroxidase and glutathione reductase, indicating antioxidant defense activation. In *M. galloprovincialis*, across digestive gland, gills and muscle tissues, the most relevant effects were linked to PS

and HDPE. HDPE clearly caused neurotoxicity in gills and muscle, and oxidative stress with antioxidant defense activation in the digestive system and gills. PS mainly affected antioxidant biomarkers, consistently in both digestive system and gills.

This study evidenced how different MPs interact across different trophic levels and environmental compartments, which is critical for a more comprehensive environmental risk assessment.

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3.18.P-Mo229 Using bivalves to assess the risks of microplastics and associated pollutants: the case of the Loire River, France

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Aquatic environments represent the ultimate sink for many contaminants, posing a risk to organisms and ecosystems. Among these pollutants, microplastics (MPs) are emerging contaminants that are still rarely integrated in risk assessment while their toxic effects towards aquatic organisms have been largely demonstrated. However, linking these effects to ecological consequences observable in the field remains a major challenge. The ability of MPs to adsorb, transport and release toxic chemicals, further complicates the assessment of their actual impacts.

To quantify the effects of MPs within a risk assessment framework, new strategies have to be developed. In this context, an innovative experimental design was set up to evaluate the Loire river contamination and its biological impact on bivalves from different habitats, including some never been employed in biomonitoring programs. The objective was to establish an integrated biomarker response (IBR) index that summarizes biomarker variations, facilitating risk assessment.

For this purpose, four bivalve species were selected: two marine bivalves (*Scrobicularia plana*, *Mytilus edulis*) and two freshwater (*Corbicula fluminea*, *Dreissena polymorpha*), encompassing both water column and sediment compartments. Individuals were placed for three weeks in plastic-free cages in the downstream of the Loire river, from Angers to Saint-Nazaire. Caging was carried out in spring and autumn 2025. Variations in the response of several selected biomarkers observed were used to develop an IBR index. Biomarker variations were correlated with chemical contamination in the sites and pollutants bioaccumulation within organisms. Results highlighted a site-dependent contamination by pollutants such as MPs, that differentially bioaccumulated within organisms depending on their habitat. In addition, differences in biomarker responses at multiple organizational levels (immunological, biochemical and molecular) observed could be correlated to the presence of pollutants.

The study demonstrates that the use of caging approach combined with an IBR determination strategy allowed consideration of both multichemical exposure together with responses at multiple levels of the biological organization. In addition, as this double approach can be applied to different environments (freshwater, marine systems), and various matrices (water column, sediment), it represents a potentially valuable strategy for ecological risk assessment.

3.18.P-Mo230 Toxicity of microplastics as single and mixture stressors to *Lumbriculus variegatus* in sediment exposures

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Sediments are sinks to microplastic particles, although most of ecotoxicity studies that have been performed so far are related to the water column. In freshwater environments, levels in sediment were orders of magnitude

higher than in water, what is a concern for biota inhabiting these environments. In our study, the toxicity of polystyrene (PS) and polybutylene succinate (PBS) microplastics and polyester (PES) microfibers was assessed using the benthic invertebrate *Lumbriculus variegatus* (Oligochaeta) as model organism. *Lumbriculus variegatus* is a widely used organism in toxicity tests and a key link between contaminants present in sediments and upper trophic levels. The materials were spiked to natural lake Höytiäinen sediment at two concentrations (0.5 and 5% of sediment dry weight), including additional food in form of *Urtica* spp. (0.5%). In addition to the single stressors, exposures to mixtures of all the materials were also set (33.33% of each material). Water parameters, including oxygen, conductivity and pH were monitored. Ten worms were added, and they were exposed during 28d following guidelines. Endpoints that were monitored at 28d were growth (dry weight) and reproduction (number of worms). These variables were compared using one way ANOVA or Kruskal Wallis methods for parametric or non-parametric data. The results obtained showed that only the PES microfibers at the highest concentrations (5%) caused an effect in both endpoints. This is in accordance with other studies that indicate a higher toxicity of microfibers compared to microparticles due to the food dilution effect. Interestingly, mixtures of the three materials did not cause harm to the worms. The gut content of the worms was also collected and will be analyzed for microplastics content. Potential degradation and formation of biofilm will be monitored with SEM. Overall, the present study adds valuable and missing information that could be used to determine the thresholds for sediment content regarding microplastics. The results could also be used to compare different materials that are widely used. They include a traditional thermoplastic, a supposedly biodegradable plastic in the form of particles and a microfiber.

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3.18.P-Mo231 Toxicological Effects of Microplastic and Nanoparticles Contaminated Fish Feed on *Cyprinus carpio* Growth and Oral/Intestinal Microbiome

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Microplastic (MP) and nanoparticle (NP) contamination in fish feed threatens aquacultural productivity and food security. In this study we assessed the effects of dietary exposure to MP, NP, and their combination (MNP) on *Cyprinus carpio* growth and oral/intestinal bacterial composition. Contaminated feed significantly reduced weight gain and feed efficiency ($p < 0.05$), with MNP causing the most severe impacts on fish weight gain. Moreover, 16s rRNA sequencing revealed a significant shift in bacterial taxa, marked by pathogenic *Aeromonas* and *Vibrio* enrichment and commensal reduction, particularly in the intestines ($p < 0.001$). Beta diversity confirmed taxonomic shifts (PERMANOVA: $R^2 = 0.248$, $p = 0.001$), with intestinal bacteria more sensitive than oral bacteria. *Aeromonas* abundance correlated strongly with growth impairment (Mantel $p < 0.05$; $R^2 > 0.5$), indicating that it is a key growth-limiting pathogen and is elevated by contaminated feed. These findings highlight the dual risk of MNP-contaminated feed that disrupts the microbiome and decreases growth performance. Strategies to mitigate these effects are urgently needed to secure aquaculture feed.

3.18.P-Mo232 Nondestructive Flowcytometric Method for the Detection and Interaction of Micro and Nano Plastics in Bonemarrow.

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Introduction:

Microplastics and nanoplastics (MNPs) have recently been identified in several human tissues, yet their presence and potential effects in bone marrow remain largely unexplored. Existing detection methods rely on destructive analytical techniques, which limit the study of MNP–cell interactions. This work describes the ongoing development of a non-destructive flow cytometric approach to detect MNPs alongside bone marrow cells.

Methods:

Bone marrow samples obtained from femoral heads were spiked with various plastic types and particle sizes. In addition, THP-1 monocytic cells were included as an exploratory control model, exposed to the same plastic to acquire cell internalization and interaction controls. Samples were fluorescently labelled and analyzed by multiparameter flow cytometry. Data processing in Infinicyt, including the use of principal component analysis (PCA), was explored to assist in separating cellular events from microplastic particles.

Results:

Preliminary findings indicate that labelled microplastics can be distinguished from bone marrow cells using combined scatter and fluorescence characteristics. Initial analyses suggest that PCA may enhance separation between particle populations, though optimization is ongoing. Early experiments using THP-1 cells provide a controlled reference context, helping assess the reproducibility of plastic-associated signatures. In addition using a mix of plastic labelling agents can assist in uniquely fingerprinting the microplastic.

Conclusion:

This non-destructive flow cytometric strategy shows promise for detecting MNPs within complex biological matrices and for probing their interactions with cells. Ongoing work aims to further refine the PCA-based analysis, expand the range of tested polymers and particle sizes, and integrate additional cellular markers, including more detailed evaluations using THP-1 and primary bone marrow populations. Which could lead to a high throughput clinical analysis method for investigating the presence and interactions of MNPs.

3.18.P-Mo233 Health impacts of microplastics and nanoplastics: consensus on research needs

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Identifying the human health risks of exposure to micro- and nanoplastics (MNPs) is an urgent priority due to first findings of MNPs in human tissues and the increasing prevalence of plastics in society. From 2021 to 2026, the European Union funded five Horizon 2020 research projects under the CUSP cluster with the aim of supporting human health risk assessment for MNPs. One output of this work is the CUSP Roadmap detailing research needs for health risk assessments of MNPs in Europe, outlining short, mid-term and long-term needs until 2032 for five transversal themes: Materials and Methods, Hazard Assessment, Exposure Assessment, Risk Assessment Frameworks, and Mitigation Measures. The CUSP Roadmap sets clear priorities for research, policy, and outreach, and provides the evidence base needed to guide regulation and innovation. It identifies the need for policymakers, scientists, and industry to work together strategically to reduce risk, accelerate the transition toward safer and more sustainable alternatives, and ultimately protect public health from the harm caused by MNPs.

3.18.P-Mo234 Microplastic fibers – double trouble in environmental monitoring

Lina Büngener, Dora Mehn, Guillaume Bucher and Douglas Gilliland, European Commission - Joint Research Centre (JRC), Italy

Ongoing standardization and regulatory activities are trying to address microplastics (MPs) in the environment, and to enable comprehensive monitoring of this pollutant of emerging concern by establishing guidelines for sampling, sample preparation and analysis. Meanwhile, it is becoming evident that MPs in the form of fibers likely will need to be looked at separately. While they are a highly abundant form of MPs in the environment, especially in atmospheric and wastewater samples, several issues surrounding sample preparation and analysis persist: Because of their dimensions, it is difficult to ensure that fibers are captured representatively during MP sampling, which usually involves sieves. MP fibers are often a major contaminant of procedural blanks.

Analysis methods depending on the automated analysis of (optical) images, such as Raman micro-spectroscopy or FT-IR micro-spectroscopy, usually have difficulties to accurately identify and measure fibers. And finally, MP fibers could exhibit a different environmental fate compared to “round” MP particles.

This presentation outlines the major challenges encountered related to the environmental monitoring of MP fibers as a particular group of MP particles, and some experimental results exploring potential solutions are presented for discussion.

3.19.A Out of the Norm: Assessing Fate, Safety, and Sustainability of Biodegradable Polymers

3.19.A.T-01 Soil Biodegradation of Biobased Polyesters: Towards Structure-Reactivity Relationships

Thijs Vangeel and Michael Sander, ETH Zurich, Switzerland

Biodegradable polymers are essential for advancing a circular plastic economy, particularly when designed to reintegrate into natural elemental cycles through industrial composting or environmental biodegradation. Aliphatic–aromatic copolyesters, such as poly(butylene adipate-co-terephthalate) (PBAT), are widely used due to their mechanical performance and biodegradability, yet they typically rely on fossil-derived monomers. Substituting these components with renewable alternatives – most notably 2,5-furandicarboxylic acid (F) – offers a pathway toward fully bio-based, biodegradable materials.

In this study, 28 fully bio-based F-containing aliphatic–aromatic copolyesters were synthesized by systematically varying the diol, aliphatic diacid, and aromatic diacid components, as well as the aromatic-acid content (30–70%). Thin films (~30 µm) were prepared and evaluated for soil biodegradability using controlled laboratory incubations (~400 vessels). Residual polymer was periodically extracted via Soxhlet extraction and quantified by ¹H-NMR, which also provided insight into compositional changes during degradation.

To disentangle the effects of molecular structure, soil type, and degradation kinetics, the experimental design was divided into three targeted subsets. Subset A assessed one representative F-containing polyester across six standard soils, benchmarked against PHBH as a positive control. Subset B examined biodegradation dynamics for nine structurally varied polyesters through five time points. Subset C compared the complete set of synthesized copolyesters in a single standard soil (LUFA 6S) at two incubation durations.

Across all subsets, clear trends emerged linking biodegradability to copolyester molecular features, including the nature of the aromatic and aliphatic diacids and the diols, as well as the aromatic-acid content. These findings establish structure–biodegradability relationships that can guide the design of next-generation fully bio-based, soil-biodegradable aromatic–aliphatic copolyesters.

Disclaimer/Disclosure:

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3.19.A.T-02 Soil Biodegradation of a Rice Dust-Based Bioplastic and Its Effects on Microbial Communities

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Soil is a major reservoir for plastic disposal, which can interfere with the biogeochemical processes, thereby impacting soil multifunctionality and productivity. Conventional plastic replacement with bioplastics, particularly those developed from low-value natural by-products, emerged as a promising strategy to tackle this global challenge, one that promotes circularity, reduces dependence on fossil fuels, lowers greenhouse gas emissions, and mitigates the impacts on ecosystems. However, assessing the effects of bioplastics on the functioning of soil ecosystems remains paramount. In this study, the impact of a rice dust-based bioplastic on soil functioning was assessed during the defragmentation stage of the biodegradation process. The experimental design comprised two treatments: i) with bioplastic (BIO) and ii) without bioplastic (CT). At each sampling time (days 0, 7, 14, 28, 42, 56, and 84), three replicates were destructively sampled, and a comprehensive set of analyses was conducted, including soil enzymatic activities, multiple soil nutrient fractions, and soil properties. The bioplastic piece was also recovered, gently cleaned, oven-dried, and reweighed to assess mass loss. Biodegradation results showed almost complete defragmentation. Model-based estimation of the half-life for Lufa 2.2 soil-incubated bioplastics was approximately 15 days. The results of soil functioning tests showed that the bioplastic induced significant changes in certain soil enzymes and nutrient fractions. The transient spike in dissolved organic carbon observed at the beginning of the experiment led to consistently higher values of microbial biomass carbon. On the contrary, soil incubated with bioplastic showed significantly less content in inorganic N (mainly nitrates) and total dissolved N, but similar values of microbial biomass nitrogen. Urease activity was significantly higher in soil incubated with bioplastic, suggesting a requirement for additional N mining by soil microbial communities. Likewise, bioplastic incubations led to reduced inorganic P and ultimately to significantly different microbial biomass phosphorus over time. Overall, the bioplastic appears to have triggered a positive priming effect, requiring additional action from the microbial community to extract native N from the soil organic matter. A similar situation appeared to occur for P. Further work should focus on structural alterations in soil microbial communities.

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3.19.A.T-03 Chemical complexity and ecotoxicological effects of leachates from bioplastic and conventional plastic products

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Bioplastics (bio-based and/or biodegradable) are often promoted as environmentally safer alternatives to conventional plastics (fossil-based and non-biodegradable), yet their chemical complexity may present ecotoxicological risks. This study compared water-based leachates and methanol-based extracts from five commercial plastic products, including conventional plastic and bioplastic bags. Polymer composition was verified via FTIR and DSC, while elemental composition was measured using ICP-MS/OES. Non-target analysis (HR-LCMS) characterized unknown organic constituents, and ecotoxicity was assessed using marine bacteria (*Aliivibrio fischeri*), freshwater algae (*Raphidocelis subcapitata*), and *Daphnia magna*.

Results showed that biodegradable plastics released a more chemically diverse array of organic compounds and, in some cases, higher concentrations of metals (e.g., Zn and Fe) than conventional plastics and non-biodegradable bioplastics. Chemical profiles differed between water- and methanol-based extractions, and products with the same base polymer exhibited distinct leachate compositions and ecotoxicity responses, emphasizing the importance of assessing finished products rather than base polymers alone. Bioplastic leachates caused stronger ecotoxic effects, particularly inhibiting algal growth by up to 80 %. In contrast, *D. magna* was sensitive only to a bio-based, non-biodegradable bag, while marine bacteria showed slightly higher sensitivity to bioplastics compared to conventional plastics.

These findings suggest that bioplastics can release organic compounds and metals at levels comparable to or exceeding those of conventional plastics, thereby challenging the assumption of their inherent environmental safety and underscoring the need for comprehensive chemical and ecotoxicological evaluations.

3.19.A.T-04 Water-soluble polymers in the aquatic environment – Lessons learned from two case studies with Polyethylene glycole and polyquaternium polymers

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The field of water-soluble polymers (WSPs) comprises neutral, anionic, and cationic polymers with estimated individual annual production volumes in the range of hundreds to hundreds of thousands of tons. Unlike conventional solid polymers for which an increasing share is recycled or incinerated, many WSP applications do not facilitate recollection. While environmental occurrence data for WSPs is exceedingly scarce first evidence indicates that this combination of high production volumes, widespread applications, and low potential for recollection can result in substantial emissions into the environment in the µg/L or near sources even mg/L range. Yet, assessment of WSPs in the environment is still in its infancy coping with severe analytical challenges and an at best rudimentary understanding of the environmental fate and effects of WSPs. Herein we compare the results from two case studies, one analyzing polyethylene glycol (PEG) a very high production volume but readily biodegradable WSP in surface water and wastewater treatment plants with another study analyzing four polyquaternium (PQ) polymers in surface water, highlighting challenges and knowledge gaps in the environmental assessment of WSPs.

PEG was analyzed for in 18 surface water samples and two wastewater treatment plants. Despite its inherent biodegradability, PEO concentrations ranged from <LOD to 11 µg/L for surface waters (11/18 samples >1 µg/L). A molecular weight fraction between 1.3 and 4 kDa was dominant in wastewater treatment plant effluent and most surface waters, likely due to molecular weight dependent sorption and biodegradation processes. Four PQs (PQ-2, 6, 7, and 10) were analyzed for 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). Due to the lack of well-characterized standards and strong interactions with humic acids accurate quantification of PQs is not possible 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, partially exceeding concentrations for which ecotoxicological effects have been reported. These results indicate that a shift towards degradable WSPs is required.

Disclaimer/Disclosure:

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3.19.B Out of the Norm: Assessing Fate, Safety, and Sustainability of Biodegradable Polymers

3.19.B.T-01 Screening Biodegradation Tests: History, Predictive Value, and Clarification of Common Misconceptions in Environmental Persistence Assessment

Drew McAvoy, University of Cincinnati, United States

Screening biodegradation tests have been a foundational component of environmental fate assessment for more than forty years, yet their purpose, limitations, and predictive power are often misunderstood. These tests originated during the regulation of surfactants in the 1970s, when regulators and industry required harmonized and reproducible methods for identifying chemicals capable of rapid biodegradation under aerobic conditions. The test development originated with surfactant regulations, and culminated in the Organization for Economic Cooperation and Development (OECD) tiered biodegradation framework established in 1981, which introduced Ready biodegradability tests, Inherent biodegradability tests, and Simulation test. These tests are now embedded in regulatory frameworks across the world.

Ready tests are intentionally designed as stringent stress tests. They rely on low, unacclimated microbial densities typically between ten thousand and one hundred thousand colony-forming units per milliliter high chemical-to-biomass ratios, and mineralization endpoints such as carbon dioxide evolution or oxygen consumption. The goal is to evaluate whether a substance possesses the inherent capacity for rapid aerobic biodegradation under conservative test conditions.

A substantial body of empirical evidence confirms the predictive value of Ready test outcomes. This includes studies of environmental half-lives, and comparative tests across sludge and river water tests. This presentation discusses frequently cited concerns such as the sixty-day duration for enhanced ready tests, the so called “missing 40%”, and perceptions on poor waste water treatment plant performance. Many of these concerns arise from the challenge of communicating a complex scientific topic in simplified form to external audiences. Recent work from both industry and academia, including environmental monitoring studies, further reinforces the historical basis for the applicability and predictability of OECD tests, even for more complex substances such as polymers and chelants. The ongoing challenge for academia is to ensure robust communication of these concepts within regulatory frameworks and risk assessments. This presentation will review the underlying science and invite discussion on how to reassure and effectively explain the science to non-scientific audiences.

Disclaimer/Disclosure:

This presentation is of the sole opinion of the author.

3.19.B.T-02 Towards an Integrative Assessment of Polymer Biodegradation

Kathleen McDonough¹, Jennifer Menzies², Ashley Wilcox², Nigel Yates², Taylor Neal², Sandra Isaacs², Spiro Stilianoudakis², Mark Sivik², Maura Hall², Kristin Connors², Susan A Csiszar¹ and Lijuan Li², (1)Procter & Gamble, Mason, United States, (2)Procter & Gamble, United States

Significant focus has turned to the development of biodegradable water soluble polymers for use in products disposed of down the drain into the sewer system. In addition, there is renewed regulatory focus on methods for assessment of polymer biodegradation as the EU Commission evaluates new approaches to register polymers under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Polymers are

composed of structurally similar molecular chains with varying molecular weights (MW) typically >1000 Da and complex structural features. Biodegradation of polymers is more complex than for lower MW molecules due to the need for extra-cellular enzymatic cleavage of the polymer into smaller oligomeric fragments that are able to be transferred intra-cellular in order for complete mineralization to occur. The high MW and complex nature of the starting test substance and the fragmentation into many additional transformation products, results in significant analytical hurdles for quantifying biodegradation potential, biodegradation pathways, and rates of primary and ultimate biodegradation in a robust manner. Limited polymer biodegradation assessments have been conducted with actual test data across different biodegradation assays (screening, simulation, novel test setups) and with inoculum from different environmental compartments to quantitatively evaluate whether a polymer is biodegradable. This research developed an integrated approach to evaluate the biodegradation potential of different complex water soluble polymers that are used in down the drain products. For each polymer under investigation, this research evaluated known biodegradation pathways and biodegradation potential based on a variety of screening and simulation type studies with mixed microbial communities from activated sludge and river water compartments. This research further explored the impact of test design (test chemical concentrations, inoculum concentration and handling) on study results. These data were assimilated to evaluate the biodegradation potential and pathway of each polymer in activated sludge wastewater treatment and freshwater compartments.

3.19.B.T-03 Stable isotope labeling in polymer biodegradation assessment: from research to regulation

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Biodegradability is a desirable trait for many structural, water-soluble, and water-dispersible polymers used in diverse applications. It ensures that polymers can undergo complete microbial metabolic utilization in natural (soil, water) or engineered (wastewater, compost) environments, forming CO₂ and microbial biomass. Biodegradability therefore is a critical safe-and-sustainable by-design criterion to prevent the formation and accumulation of persistent polymers, including stable micro- and nanoplastics, in the environment.

Biodegradation is commonly assessed by incubating polymers in the desired matrix (e.g., soil, compost, sludge inoculum) coupled with respirometric analysis of polymer-derived CO₂ formation (i.e., mineralization). While such analyses provide direct evidence for biodegradation, several interpretational challenges remain. First, standard mineralization measurements can be affected by ‘priming’, a substrate-dependent artifact for which substrate-free control incubations cannot fully correct. Second, incubations often need to be conducted at high polymer concentrations to generate sufficient signal above background CO₂ formation from natural organic matter (e.g., for compost). Alternatively, the background is diluted and/or enrichment inoculae need to be used for incubations (as in the case of sludge). Third, the non-mineralized fraction of the polymer carbon cannot be quantified as residual polymer carbon cannot be delineated from background natural organic matter carbon in the incubation matrix. Therefore, mass balances on polymer carbon cannot be closed. Finally, the relative contributions of residual polymer and microbial biomass derived from polymer carbon remain uncharacterized. This contribution demonstrates how the above challenges can be addressed and new fundamental insights into biodegradation obtained using stable isotope-labeled polymers in incubation studies. The interpretational power of this approach is illustrated through selected studies with ¹³C-labeled structural and water-soluble polymers. Beyond highlighting its value for academic research, the contribution discusses the unique role of stable-isotope labeling in future regulatory assessments of polymer biodegradability, noting commonalities with, but also advantages over, radioisotope labeling approaches.

3.19.B.T-04 Biodegradability of Polylysine in Wastewater: Identification of Key Microbial Degradors and Enzymatic Mechanisms

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Polylysines are widely used in personal care formulations due to their antimicrobial activity, film-forming ability, and moisturizing properties. To further develop this versatile class of polymers, and since they are released into wastewater streams, it is important to understand their biodegradability in this environment. In this study, we aimed to identify key microbial degraders capable of utilizing polylysine as a sole carbon source. Therefore, we established an enrichment cultivation protocol using an activated sludge sample from a wastewater treatment plant. Subsequently, a metagenomic analysis was conducted to identify candidate microbial degraders that were enriched during the cultivation. Selected microbes were obtained from public microbial collections and modified standard biodegradation tests confirmed the ability of these strains to mineralize polylysine into carbon dioxide. A proteomics analysis performed at specific time points during the

biodegradation test revealed key enzymes implicated in polylysine degradation. These findings advance our understanding of polylysine biodegradation and may inform future strategies to mitigate bioaccumulation in wastewater environments.

3.19.B.T-05 Towards Holistic Approaches to Modeling Polymer Biodegradability

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A key aspect of sustainable innovation involves designing polymers that can biodegrade within their receiving environments. An important element in assessing, understanding, and forecasting biodegradability is the application of computational tools. While there are established methods for predicting the biodegradability of small molecules, the development of models for polymers is still in the early stages. Additionally, there are several methods to assess and measure polymer biodegradation in aqueous media such as (but not limited to) enzymatic assays, screening studies, simulation studies, all of which provide a range of insights from impacts of increasing environmental realism and enzymatic and microbial transformation pathway and kinetic information. Furthermore, adaptation of these methods to polymers is an active research area, allowing for greater availability of insights and data to build predictive and understand biodegradation mechanisms. As such, a holistic framework to modeling polymer biodegradability is recommended that leverages both mechanistic, kinetic, and data-based approaches that can be integrated across experimental assays and studies. Mechanistic and kinetic modeling techniques can be used, for example, to link and understand differences across assay results. Data-based approaches such as machine learning can integrate structural descriptors of polymers with experimental results, and can be combined with the mechanistic-based approaches. A practical starting point for building machine learning models leverages screening biodegradation assay results where there is currently sufficient existing data to build frameworks and models, which can then be extended to other assay types. As part of the holistic modeling approach, we present a machine learning model that leverages data from polymer screening biodegradation studies and recent advances in polymer informatics. The approach includes a framework for consistent polymer representation across several water soluble polymer classes including copolymers, allowing for data across classes to drive predictions. The model was built using publicly available data and was evaluated using an independent test set of recently generated screening biodegradation data.

3.19.P Out of the Norm: Assessing Fate, Safety, and Sustainability of Biodegradable Polymers

3.19.P-We268 Improved Digital Representation of Polymers for Better Biodegradation Assessment

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A key tool in the in-depth assessment of how polymers break down in the environment will be the use of predictive modelling. For the assessment of small molecules, it has proven an invaluable addition to standard lab-based methods, and can give additional insights into breakdown products, toxicity, pathways and enzyme binding. The advent of more complex modelling techniques and supercomputing capacity, together with the slow expansion of openly available datasets has allowed many accurate models to be developed which meet specific requirements of regulators (for example the recent QSAR Assessment Framework guidelines). The relative simplicity of describing small molecules to computers here has also facilitated such development.

The development of accurate predictive models specifically for polymer biodegradability is however an altogether more complex hurdle. One of the main issues contributing to the absence of accurate models here is a lack of accurate representation methods to describe polymer structure. Traditional techniques fail to include structural polymer properties which are important for development of accurate models. Although many techniques are already available which describe the chemical nature of polymers (repeating units, end groups, branching, etc), none so far have been able to accurately encompass the stoichiometric nature of polymers; namely, molecular weight distribution and wider polymer microstructure. For prediction of biodegradation, it is crucial this information is included, as so many of these processes are directly affected by such information – it is widely understood that increased molecular weight and crystallinity affect biodegradation due to uptake and accessibility constraints.

Here, we will present a new advanced polymer representation method which comprises chemical as well as stoichiometric data. This method is based on the G-BigSMILES techniques outlined by Schneider et al in 2024.

With the additional stoichiometric data, we further propose how this can be used to accurately represent real-life polymers, and how this can be applied to biodegradation prediction. By offering better, more accurate predictions of how polymers biodegrade in various environments, we hope that this can further the discussion on how fate of polymers can be better assessed.

3.19.P-We269 Water Soluble and Dispersible Polymer Biodegradation and Transformation Pathway Data Curation to Enable the Development of Modelling Tools

Susan A Csiszar¹, Marlies Bergheim Sr M², Harald Streicher³, Glauco Battagliarin⁴, Jessica Mugleston⁵, Guillaume Cottin⁶, Michael Sander⁷, Jasmin Hafner⁸, Thomas Hofstetter⁹, Kathrin Fenner¹⁰, Ryan Heisler¹¹ and Amelie Ott¹¹, (1)Procter & Gamble, Mason, United States, (2)Corporate Scientific Services, Henkel AG & Co. KGaA, Germany, (3)Beiersdorf AG, Germany, (4)Research Biopolymers, BASF, Germany, (5)BASF, United Kingdom, (6)L'Oréal, France, (7)ETH Zurich, Switzerland, (8)Environmental Chemistry, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (9)Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland, (10)Environmental Chemistry, University of Zürich, Switzerland, (11)International Collaboration on Cosmetics Safety (ICCS), United States

We present a framework for a new initiative from the International Collaboration on Cosmetics Safety (ICCS) to enhance the development of in silico tools and methods for polymer biodegradation. Modeling and computational tools for estimating polymer biodegradation are key aspects to better understand, predict, and support grouping, weight-of-evidence, and read-across assessments of the biodegradability of polymers. While there are several modeling techniques available for small molecules, models for polymers are still in the early stages, and model development relies on experimental data to build the models and to evaluate them. This project emphasizes the collection, curation, and assessment of experimental data related to polymer biodegradation, including transformation pathways and enzymatic assays, to inform the development of robust predictive models. By addressing identified gaps in data reporting and availability, this initiative seeks to facilitate the creation of a comprehensive database, prioritize modeling methodologies, and highlight future data generation needs. The focus is on water-soluble and dispersible polymers within environmentally relevant compartments, particularly wastewater treatment and aqueous environments, to support the ongoing ICCS collaborative research activities in polymer biodegradation.

3.19.P-We270 High Throughput Automation Modifications to OECD 302B Biodegradation Assay Using Robotic Interface

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The OECD 302B (Inherent Biodegradability: Zahn-Wellens/ EVPA Test) is a screening test for inherently biodegradable materials that uses activated sludge as an inoculum source and measures the biodegradation through analysis of dissolved organic carbon (DOC). This assay is a powerful tool for analyzing the fate and biodegradation potential of both small molecules and polymers used in various applications, including pharmaceutical, personal care, and industrial products that are released into domestic or industrial wastewater treatment facilities. In this assay, samples must be taken from the bottle at the sampling timepoint, filtered, and then analyzed with a total carbon analyzer (TOC) or CHNS machine. This process can be labor intensive and time consuming and therefore may be a limiting factor for the test material numbers that can be analyzed during a given trial. To mitigate this issue and make this assay higher throughput, a robot was designed in collaboration with Synchron, Etten-Leur, Netherlands. The setup for this system is five trays of 12 test bottles, arranged in a row along a lab bench. There is an overhead track on which the robotic sample arm moves down the row. The automated series for sampling is as follows: (1) The robot uses first a pH probe to record the pH into its internal computer; (2) the robot picks up a sample from the test bottle and then a filter (3) the filtrated sample is deposited into a test tube; (4) the filter is released into a disposal area (5) the needle is rinsed and cleaned; and (6) the bottle is refilled with deionized water to its original amount. The study operator will then take the prepared test tube samples and be able to place them directly to the TOC for analysis of the carbon content by TOC measurements. The OECD 302B analysis method is now fully operational by a robotic interface enhancing degradation measurements on a daily basis with low labor cost and improved high throughput capabilities.

3.19.P-We271 Improving Polymer Biodegradability Assessments with the Probabio Method

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Introduction

Water-soluble and water-dispersible polymers are essential in many formulations, such as Home and Personal Care and agricultural products. As sustainability becomes a priority, accurate biodegradation assessment methods are needed. Current OECD guidelines, designed for simple substances, do not fully address the environmental fate of complex polymers, which often degrade via pathways not covered by standard tests.

Objective

This study evaluates polymer biodegradation under diverse biotic and abiotic conditions, aiming to improve classification and labeling of polymer ingredients based on biodegradability.

Materials and Methods

Five polymers were tested under 400+ conditions. Standard OECD tests (301, 306, 302) were performed in various environments: wastewater treatment plants (WWTPs), freshwater, soil, seawater, and sediments. Inoculum sources included:

WWTPs: La Roche-sur-Yon (80,000 PE), Bellevigny (4,000 PE)

Lagoon: Bellevigny (250 PE)

Soils: organic and intensive (La Roche-sur-Yon)

Freshwater: l'Yon, La Loire

Sediments: Loire

Seawater: Atlantic coast

Tests used two temperatures (12°C, 24°C) and two inoculum concentrations (standard and 10x higher).

Experimental Design

A Design of Experiments (DOE) approach, Probabio, was used to systematically vary biotic (inoculum composition, diversity, density) and abiotic (chemical properties, exposure) factors, reflecting environmental heterogeneity.

Results and Discussion

Biodegradation was measured at 28 and 60 days. Key findings:

Inoculum Composition/Density: Presence and diversity of degraders were critical.

Inoculum History: Pre-exposure to the polymer or similar compounds enhanced degradation.

Chemical Properties/Exposure: Polymers with greater environmental exposure had higher biodegradation due to pre-adapted microbes; novel substances degraded less.

Lab vs. Environment: Laboratory conditions (e.g., Food-to-Mass ratios) often differed from real environments.

Stochastic Variability: Sampling differences affected results, highlighting the need for representative samples.

Conclusion

Biotic and abiotic factors significantly influence polymer biodegradation. Using a comprehensive DOE framework, this study proposes a refined labeling system: readily biodegradable, enhanced ready, ultimately inherent, or not biodegradable. This approach supports improved environmental assessment of complex polymers, aligning with sustainability goals.

3.19.P-We272 Investigating Freshwater Microbial Communities and Their Potential for Biodegradation of Water-Soluble Polymers

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Water-soluble polymers (WSPs) are providing key functions in numerous consumer products, including those used for home and personal care. After use, WSPs can enter natural aquatic environments (e.g. rivers) – mostly via wastewater treatment plant effluents. Biodegradation is a key process controlling the fate of WSPs in these systems and biodegradability a key desired feature of sustainable WSPs. Standardized biodegradation screening tests are typically performed under closed-bottle conditions over several weeks. The results of such tests can vary considerably depending on the composition of the sampled microbial community used as inoculum. Moreover, closed incubation can induce community shifts, sometimes referred to as “bottle effects”, as microbial communities adapt to confined conditions and resource limitations. Grazing by eukaryotes can further influence these dynamics by selectively removing certain bacterial taxa, thereby shaping community structure and potentially altering biodegradation outcomes. Understanding the variability of sampled microbial communities and the shift of these communities during laboratory biodegradation testing is important for further

improving biodegradation tests with respect to reproducibility and environmental relevance. In this study, we analyzed the composition and dynamics of riverine microbial communities sampled for WSP biodegradation testing using 16S rRNA gene amplicon sequencing. Water samples were sampled at different locations along the Danube. Microbial community shifts were monitored during incubation experiments to examine how natural communities change under closed-bottle conditions typically used in biodegradability testing. Furthermore, we assessed the impact of eukaryotic grazers on bacterial community dynamics during these incubations. Selected WSPs (i.e., polyamino acids, polysaccharides, polyethylene glycol, and polyvinyl alcohol) were incubated with freshwater microbial communities and biodegradation was assessed in respirometric laboratory tests. Together, our experiments provide insights into the natural and induced variability of freshwater microbial communities and their potential to biodegrade WSPs in riverine systems.

Disclaimer/Disclosure:

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3.19.P-We273 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 CMC with different degrees of substitution will be synthesized. 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 was already 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.

3.19.P-We274 Inter-laboratory testing of two polyurethane dispersions according to the enhanced OECD 301 F test guideline

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Biodegradability of polymers is a desirable trait to prevent long-term impacts to the environment. Furthermore, degradability is an important consideration in regulation of polymers, such as within the EU REACH restriction on synthetic polymer microparticles. Polyurethane dispersions (PUDs) are polyurethane polymer resins dispersed in water and are used in a variety of applications, including agriculture (e.g. as binders for coatings, seed treatment adjuvants and others). In agricultural applications, environmental release of polyurethanes is

effectively inevitable, via deposition of treated materials onto soils and plants. In line with the principles of safe and sustainable by design (SSbD), modifications such as for the backbone and segment architecture (e.g. cleavable motifs, altered crosslinking) allow for adjusted chemical properties and also largely influence their biodegradability potential. An inter-laboratory comparison was performed on two PUDs to assess their biodegradability according to the enhanced OECD 301 F test guideline. The ring test involved six laboratories from four countries and followed a specific protocol to investigate inter- and intra-test variability. Tests included microcrystalline cellulose as an additional positive control and three replicates per treatment. Test results indicated that both PUDs were extensively biodegradable, with biodegradation exceeding 60% after either 28 days or 60 days in all labs. The first test item was slower to degrade than the second, but both polymers showed similar biodegradation extents by day 60. Considerable variability in the evolution of biodegradation between replicates was observed, although results converged within an acceptable range by the end of the test. The results of this inter-laboratory comparison demonstrate the potential variability between replicates and across laboratories when conducting ready biodegradability testing of polymer dispersions, and the importance of extended test durations. Preliminary results of investigations into inoculum characterisation, preservation and re-use will also be presented.

3.19.P-We275 Evaluating Analytical Tools for Assessing the Degradation of Polysaccharide-based water-soluble polymers

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Biodegradable polymers are promoted as alternatives to poorly degradable materials, yet their environmental fate and transformation processes remain insufficiently understood. Their high molecular weight and structural diversity make them analytical outliers that often fall outside standard biodegradation tests, highlighting the need for methods tailored to characterising degradation pathways and intermediates.

This study develops and evaluates a multi-technique workflow to quantify and interpret the enzymatic degradation of polysaccharides with varied chemical modifications and degree of substitutions (DS).

Unmodified pullulan and dextran, along with modified carboxymethyl cellulose (CMC) with DS from 0.6 to 1.2, were degraded in assays using substrate-specific enzymes to compare their relative degradation extent and pathways.

Degradation was assessed using complementary analytical approaches. Thin-layer chromatography (TLC) enabled rapid qualitative detection of degradation intermediates, while Ultra-High Performance Liquid Chromatography coupled with Charged Aerosol Detection (UHPLC-CAD) enabled quantitative detection of mono/di saccharides. UHPLC-CAD offered improved sensitivity and reproducibility, with detection and quantification limits of 15 and 20 µg/mL.

The workflow resolved key degradation steps and revealed that higher substitution reduced enzymatic efficiency in converting CMC into glucose, cellobiose, and celotriose. These results will be compared to published OECD 301 and 314B data of the same materials. Pullulan and dextran showed greater conversion into glucose and related intermediates, indicating the method's applicability to other polysaccharides.

Additional methods, including infrared spectroscopy, derivatisation-based mass spectrometry (MS), are being optimised to improve detection of intermediates in complex matrices. Together, these approaches provide a scalable framework for evaluating the biodegradable potential of polysaccharide polymers and their transformation products, supporting stronger interpretation of standard biodegradation tests. Scalability can be achieved through a tiered strategy, utilising TLC-UHPLC-CAD for quantification in high-concentration assays and integrating sensitive methods such as MS to characterise trace intermediates in environmentally realistic samples. Overall, this work provides a robust analytical approach that informs biodegradable polymer design and refinement of fate assessment strategies.

Disclaimer/Disclosure:

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3.19.P-We276 Rapid Screening Tool to Assess Likelihood of Degradation for Research Samples

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The demand for non-persistent polymers with a lower carbon footprint has accelerated the search for new bio-based biodegradable polymers that meet or exceed the performance over existing incumbents. Where the performance of natural biopolymers falls short, researchers have focused on designing new scaffolds and backbone modifications to maintain or introduce biodegradability while meeting performance criteria. Obtaining biodegradability measurements is a critical step of this design cycle, yet traditional screening tests (e.g., OECD 301 Test for Ready Biodegradability) take 28–60 days and are too slow for rapid innovation. We have now developed a higher throughput miniaturized assay to provide researchers faster feedback and enable prioritization of incoming samples in full scale tests. While this test cannot quantify percent biodegradability, it serves as an excellent starting point to rank molecules of interest based on their likeliness to degrade. Furthermore, this assay can provide researchers insights into the impact chemical modifications are having on biodegradability in as early as five days.

3.19.P-We277 Bridging Laboratory and Environmental Relevance: Soil Biodegradation of Polymers via Oxygen Consumption Monitoring (ISO 17556)

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The environmental fate of biodegradable polymers is a growing concern for both regulators and industry, requiring reliable and relevant assessment methods. This work presents a comprehensive methodology for evaluating polymer biodegradability in soil, developed in alignment with ISO 17556:2019. The approach utilizes indirect measurement of microbial activity by monitoring oxygen consumption in soil microcosms with pressure sensors. Changes in pressure and CO₂ capture are continuously recorded, providing a sensitive, real-time quantification of oxygen uptake by soil microorganisms as they mineralize test polymers.

Indeed, OECD 301 and 302 are considered screening tests, meaning they are first-tier assays that are easy to perform, cost-effective, and suitable for upscaling. Their main advantage lies in their stringency: they are highly selective and generally provide conservative biodegradation profiles. Thus, when a substance is classified as readily biodegradable according to these tests, it is generally assumed that it is unlikely to persist in the environment.

OECD 302 tests, which assess inherent biodegradability, further enhance the identification of a substance's potential for biodegradation or mineralization. However, they still lack environmental representativeness. In summary, while these screening tests efficiently address biodegradability criteria, they do have certain limitations regarding their environmental relevance. This is why, for second-tier testing, it is important to focus on the potential for biodegradation under actual environmental conditions, particularly by considering the impact of the matrix (e.g., soil or sediment) on the biodegradation process.

The soil test bridges this gap by simulating realistic soil conditions, enabling the assessment of polymer mineralization and persistence in a more environmentally relevant context. The protocol is versatile, accommodating various polymer types including (i) non-soluble microplastics, (ii) hydrosoluble polymers in formulations (iii) and adsorptive polymers as well as different soil matrices. It supports extended incubation periods for comprehensive monitoring. The data generated provide critical insights for regulatory submissions, product development, and sustainability assessments.

By advancing our understanding of polymer biodegradation in soil, this methodology supports Syensqo's mission to drive sustainable innovation and create solutions that benefit both humanity and the planet.

3.19.P-We278 From Functional Lifetime to Soil Breakdown: A Field Realistic Degradation Method

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From Functional Lifetime to Soil Breakdown: A Field Realistic Degradation Method

Biodegradable plastics are routinely assessed either by artificial weathering without soil contact or by standardized biodegradation assays that require shredding and intimate mixing with a test substrate. Neither approach reflects the coupled action of solar radiation, soil microbiology, and moisture cycles under use, nor do they capture the critical window in which abiotic damage (e.g., photo oxidation, hydrolysis, embrittlement) triggers loss of function long before complete mineralization. To close this methodological gap, Fraunhofer UMSICHT has developed a non standard, yet rigorously controlled, degradation test that simultaneously exposes intact specimens to real soils, sensor controlled moisture regimes, and adjustable UV irradiation. A modular rig allows six parallel trials with independent registers. UV lamps there while address two registers each. Watering is solved via drip irrigation and magnet valves maintain setpoints. Soil temperature, UV dose and ambient conditions are logged continuously. This design enables reproducible comparisons across materials

and conditions while preserving application relevant interfaces and synergies.

We will present the method and its application to BioVlies, a project targeting bio-based, biodegradable nonwovens for crop protection as alternatives to polypropylene nonwovens. Although initially developed for mulch films, the protocol readily transfers to plates and nonwovens. Within BioVlies, polymer blends identified for spinnability and mechanical performance are processed into staple fiber nonwovens. Our degradation assay complements these trials by aging the samples in a laboratory controlled, similar to real field setting. This aging gives more detailed ideas and information how biodegradable plastic nonwovens perform within their application purpose.

By providing actionable metrics that connect application performance with degradability in soil, the assay supports eco design and offers an evidence base for future standardization. We argue that field realistic, non standard testing is essential to minimize microplastic release during use while ensuring agronomic efficacy, and we invite dialogue on integrating such methods into certification and product development workflows for biodegradable plastics.

Disclaimer/Disclosure:

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3.19.P-We279 From Fragmentation to Complete Biodegradation: Understanding the Fate and Timescales of Biodegradable Polymer Fragments in the Environment

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The environmental impact of plastic pollution has driven the development and adoption of biodegradable polymers, particularly for applications where post-use recovery is impractical. The loss of the structural integrity of these materials, however, is an inevitable part of the biodegradation process and leads to an interim formation of fragments. Here we present a comprehensive investigation into the fragmentation and biodegradation behavior of both industrially compostable polymer blends and certified soil-biodegradable mulch films, integrating advanced experimental techniques with mechanistic modeling.

In several independent biodegradation studies, various biodegradable polymer blends were subjected to industrial composting (ISO 14855) or soil biodegradation (ISO 17556). Fragmentation pathways were elucidated using fluorescence, FTIR and Raman microscopy. The results revealed that blend morphology, phase separation, crystallinity, and microbial colonization are key factors influencing fragmentation kinetics. Notably, fragmentation is not merely a surface phenomenon but is driven by inhomogeneities within the bulk material and by the accessibility of degraders. The experimental data were used to parameterize the open-source FRAGMENT-MNP model, enabling the prediction of fragmentation and dissolution rates, fragment size distributions, and the conversion of polymer mass into CO₂ during biodegradation. It was demonstrated that fragmentation is a transient phase: particle counts peak and then decline as mineralization proceeds, with over 90% of polymeric carbon ultimately converted to CO₂. Modeling confirmed that residual fragments do not persist as microplastics, with predicted concentrations dropping below one particle per gram of soil within 600–700 days.

Overall, these studies provide mechanistic and quantitative evidence that certified biodegradable polymers, when properly designed and tested, do not contribute to persistent microplastic pollution. The integration of experimental and modeling approaches offers a robust framework for guiding polymer design and regulatory assessment, supporting the transition to more sustainable plastic use in agriculture and packaging.

3.19.P-We280 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 introduces a novel, integrated approach combining accelerated abiotic degradation pathways with advanced analytical techniques and standard biodegradability tests. Our aim is to identify specific degradation products, thereby elucidating a polymer's biodegradability mechanisms and a potential rapid screening test for polymers.

Polyhydroxybutyrate (PHB) and polylactic acid (PLA) served as model biodegradable and non-biodegradable polymers, respectively. To simulate and accelerate relevant hydrolytic degradation processes, polymer particles (1-10 µm) underwent hydrothermal aging at 130°C for various durations (1-120 hours). Following the abiotic treatment, the resulting water-soluble degradation products were analyzed directly by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) to characterize the initial breakdown products.

Complementary OECD 301F biodegradability tests were conducted on pristine PHB and PLA. To comprehensively analyze degradation products from these biotic tests, a study of three selected Solid Phase Extraction (SPE) cartridges (Oasis HLB, C18 and StrataX) was employed to optimize the sample preparation and handle the analytically complex biodegradability medium before LC-HRMS analysis. The feasibility of employing Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) was also explored, providing preliminary insights into identifying additional degradation products complementary to LC-HRMS.

This study introduces a novel approach, deploying applied analytical and sample preparation methods to successfully identify relevant polymer degradation products generated by the herein presented abiotic degradation method for the selected polyesters, notably correlating with those found in biodegradation media. Building on these promising findings, with our upcoming work we aim to further develop a suitable MALDI-TOF method. In parallel, our work intends to focus strategically on the in-depth exploration of novel abiotic techniques, particularly promising hydrothermo-oxidative conditions with the aim of mimicking observed biodegradation kinetic rates. These combined efforts are aimed at refining a potential predictive screening approach for polymer biodegradability.

3.19.P-We281 Assessing aqueous photo-degradability of novel polymers containing hydrolysable additives or oxidizable functional groups

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Novel polymer materials designed to enable aqueous (photo)degradation pathways have been developed at Carnegie Mellon University through an NSF ECO-CBET team project. The central hypothesis we are exploring is that aqueous (photo)degradation pathways enabled by incorporating additives or labile functional groups will then enable biodegradation pathways not possible for commodity polymers. These new materials include polymers conjugated to graphene oxide, containing protein fibril additives, or oxidizable disulphide functional groups. We have developed accelerated aging methods to evaluate the degradability of these new materials under aqueous environmental conditions involving photo-oxidation or hydrolysis transformation pathways. The degradation rate and alteration of the original monomer or polymer material is evaluated along with determining the soluble transformation products and microplastic particles produced. LC-MS/MS, GC-MS, NMR, and Raman spectroscopy are the main analysis methods being deployed. Graphene oxide (GO) was found to produce significant singlet O₂ under UV illumination, a powerful selective oxidant that can drive oxidation of the polymer material covalently bound to the GO. Graphene oxide also enables aqueous degradation pathways. Protein fibril additives should enable aqueous degradation pathways of the composite material through oxidation or hydrolysis. The disulfide functional groups incorporated into polyethylene are vulnerable to oxidation by singlet oxygen and hydroxyl radicals, producing sulphonic acids that open new degradation pathways of the polyethylene. Molecular probe experiments were used to quantify the photo-oxidants produced by the polymer materials themselves (in the case of GO) or by chemical reagents representative of common environmental precursors (H₂O₂, HCO₃⁻, NO₃⁻).

3.19.P-We282 Development and Validation of a Laboratory Protocol to Assess Biodegradability and Ecotoxicity of Per- and Polyfluoroalkyl Substance Alternative Polymers and Their Transformation Products

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Per- and polyfluoroalkyl substances (PFAS) have been extensively used due to their chemical stability and surfactant properties. However, these same characteristics also contribute to their extreme persistence, leading to their widespread occurrence and accumulation in aquatic ecosystems, and increasing regulatory scrutiny. In response, research efforts toward PFAS-alternative polymers have accelerated, with a focus on materials that

can retain performance characteristics while offering improved environmental profiles, particularly reduced persistency.

Despite this progress, standardized methods to evaluate the environmental safety of these novel polymers remain limited. While assessing the degradability of PFAS-alternative polymers is critical, there is also a need to understand whether the transformation products formed during degradation might cause ecotoxicological effects on non-target organisms.

This study aims to develop and validate a reproducible laboratory protocol for evaluating the biodegradability and ecotoxicity of PFAS-alternative polymers. Biodegradation was monitored using a ready biodegradation test based on the standard Organisation for Economic Co-operation and Development (OECD) guideline 301.

Transformation products generated during the BOD test were subsequently isolated by testing out and optimising a variety of separation techniques. The ecotoxicity was assessed using the *Daphnia magna* immobilization test (OECD 202) and the algae growth inhibition test (OECD 201), to evaluate ecotoxicological responses at different trophic levels. The protocol allows quantifying changes in ecotoxicity between the parent compound and its transformation products.

Our research contributes a practical, scientifically grounded testing framework for early-stage environmental screening of fluorine-free polymeric PFAS alternatives. By integrating biodegradation and ecotoxicity endpoints, this protocol supports the advancement of Safe and Sustainable by Design (SSbD) innovation in fluorine-free chemistry.

3.19.P-We283 Comparative Ecotoxicological Assessment of Microplastics Derived from Biodegradable and Conventional Plastics

Norihisa Tatarazako, Ryotaro Kanefuji and Yukiyo Okazaki, Ehime University, Japan

Marine pollution caused by plastics remains a critical global environmental issue.

Biodegradable plastics, which ultimately decompose into water and carbon dioxide in the environment, are considered a promising solution to the plastic waste problem. However, challenges such as limited recyclability still hinder their widespread adoption.

In our previous work, we proposed a practical ecotoxicological assessment method for intermediate degradation products of biodegradable plastics, referencing ISO 5430 (Ecotoxicity test for marine biodegradation) while aligning the procedure with Japan's chemical regulatory framework.

In the present study, we compared the ecotoxicological effects of microplastics (MPs) generated from both biodegradable and conventional plastics using acute toxicity tests with *Daphnia magna*. Direct toxicity of MPs alone was not detected; therefore, we investigated changes in toxicity when MPs coexisted with environmental pollutants. Zinc and pyrene were selected as representative contaminants. Polyethylene was used as a conventional plastic, whereas PBHB, PGA, PCA, and PBA were used as biodegradable plastics.

The results indicate that differences in pollutant adsorption capacities among plastic types alter the toxicity profiles during co-exposure. These findings highlight the importance of considering interactions between MPs and environmental contaminants when evaluating their ecological risks.

Disclaimer/Disclosure:

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3.19.P-We284 Ecotoxicity of Biopolymers – Learnings from the Lab to the Field Scale

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Biopolymers are considered as sustainable alternatives to synthetic, non-degradable polymers for use in agrotechnical products. Therefore, biopolymers remain unregulated, whereas synthetic microparticulate polymers got restricted under EC 2023/2055. However, ecotoxicity data supporting the exclusion of biopolymers from regulation is missing. This three-year study investigated: I) Whether biopolymers cause ecotoxic effects potentially linked to their substance properties, II) Which ecotoxicological methods best assess biopolymer ecotoxicity and III) The suitability of laboratory tests for predictions on the field scale (laboratory vs. outdoor

field study).

Four aquatic (e.g. OECD TG 201, transcriptomic analysis of fish embryo) and seven terrestrial test methods (e.g. ISO 15685, OECD TG 222) were selected to examine the ecotoxicological responses of six biopolymers and modified natural polymers (e.g. alginate, chitosan, CMC). The dataset generated was used to identify sensitive methods based on a LOEC (Lowest Observed Effect Concentration) comparison as well as OECD TG 216 threshold ($\pm 25\%$ deviation of treatment to control activity) analysis for soil microbial functional tests. To validate laboratory tests for field scale predictions, the identified sensitive test methods were applied in a one-year laboratory and outdoor field study (single substances/ substance mixtures).

Biopolymer exposure impaired microbial-driven soil cycles, such as potential nitrification (ISO 15685) and extracellular enzymatic activities (ISO 20130), with both inhibitory and stimulatory effects $>25\%$ depending on substance and concentration. Collembola and earthworm-related endpoints were mainly unaffected. Aquatic test results indicated that physicochemical properties (e.g. solubility, ionic charge) can be linked to the ecotoxicological outcome of the test.

For the laboratory and outdoor field study, ISO 15685 and ISO 20130 were performed. Extracellular enzymatic activity data showed a correlation between lab and field incubated samples, while potential nitrification activity correlated poorly over time. This could be linked to the degree of specialization of the microbial groups relevant to maintaining the soil functions.

Overall, biopolymers can cause ecotoxicological responses and should not be excluded from regulation without further evaluation. Incorporating soil microbial functional tests alongside aquatic tests is recommended for a comprehensive polymer assessment.

3.19.P-We285 Ecotoxicity assessment of Seaweed-Based Packaging Materials using *Capitella teleta*

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Biodegradable and bio-based materials, such as those derived from seaweed, corn, cellulose and other natural polymers, are being developed as sustainable alternatives to petroleum-based plastics. However, their increased use has not been matched by in-depth research on their environmental fate and potential ecotoxicological risks. To address this knowledge gap, this study evaluated the impact of brown seaweed-based single-use food packaging materials on *Capitella teleta*, a marine invertebrate often used as a relevant model organism for assessing ecotoxicity. The first part of the work focused on the characterisation of commercially available brown seaweed-based single-use boxes using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and contact angle. The data provided insights into the surface chemistry, morphology, microstructure, and hydrophobic character of these materials. In the second part, exposure experiments with *Capitella teleta* were done using two approaches: 1) disc material (3 mm) and 2) crushed fibre (sieved to 1000 μm). The experiments were conducted over six weeks by incubating 120 mg of samples in 12g of sediment in the artificial seawater. The measured endpoints included initial adult count (number of males and females), adult survival during each sift, time to sexual maturity, time to first reproduction, number of reproductive events, eggs per brood tube, and stages of the eggs produced in the brood tube.

Key findings from the chemical characterisation using FTIR confirmed the functional groups, as expected, while SEM imaging provided evidence of the surface coating. Contact angle measurements indicated a highly hydrophilic nature. A series of experiments was also conducted to obtain statistical data, and these provided evidence of uniformity in the coating of the commercial boxes and confirmed high data reproducibility. Ecotoxicity tests with *Capitella teleta* offered preliminary insights into the impact of these materials. This work contributes to the broader understanding of the environmental fate and potential risks associated with seaweed-derived packaging materials, supporting evidence-based evaluation of their suitability as sustainable alternatives to petroleum-based plastics.

Disclaimer/Disclosure:

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3.19.P-We286 Development and demonstration of sustainable bio-based materials for the marine environment

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Polymer-based applications are increasingly used across marine sectors, yet their fossil-fuel-based origins are considered unsustainable. The SeaBioMat project explores bio-based alternatives that could replace conventional materials while maintaining their performance. Building on earlier findings that PLA-flax composites perform well and generate fewer microplastics than polypropylene, SeaBioMat aims to advance these materials to higher TRL levels and explore additional bio-based and biodegradable polymers for composite and textile use. The project considers the entire life cycle and potential environmental interactions, such as microplastic formation and its effect on the marine environment, to ensure a lower ecological footprint. A key research task is to assess micro- and nanoplastic generation from the degraded materials, their effects on marine organisms, and material behaviour under realistic environmental conditions. To properly understand these degradation processes, the newly developed materials will undergo accelerated ageing under controlled but representative marine conditions, where extrinsic factors such as water, oxygen, microbial activity, mechanical abrasion, and UV radiation will be decoupled. Ageing will be followed by detailed mechanical and physicochemical characterisation, including tensile testing, crystallinity and molecular-weight analysis, FTIR/RAMAN spectroscopy, and gravimetric measurements. Within this framework, we will assess the potential impacts of the developed materials, focusing on their durability, degradability, and ecotoxicological effects compared to current synthetic-based materials. We will specifically focus on understanding how exposure to UV radiation, mechanical stress and temperature may lead to the formation of micro- and nanoplastics, and on determining the potential toxic effects of these microplastics on aquatic organisms such as microalgae, and benthic invertebrates such as copepods and bivalves. Microplastics generated during ageing and abrasion will be analysed using RAMAN/FTIR spectroscopy and Nile Red staining, and their ecotoxicity will be assessed through targeted bioassays. Leaching of substances from bio-based materials and their ecotoxicological impacts on the marine environment will also be studied. The expected outcomes will provide valuable insights into the environmental compatibility of bio-based polymers and support the development of sustainable materials for marine applications.

3.19.P-We287 Biodegradable Safe and Sustainable by Design Polymers for Packaging of Oily Food and Cosmetic Products

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Biodegradable polymers offer promising alternatives to conventional plastics, yet their adoption requires robust methods to assess safety, environmental fate and overall sustainability. The E-OILÉ project addresses this need by developing and validating new biodegradable materials for monodose packaging of oily foods and cosmetic products while applying a fully integrated Safe and Sustainable by Design (SSbD) framework. The project focuses on understanding regulatory requirements, identifying data needs, and ensuring that new materials comply with safety, performance and environmental criteria across their entire life cycle.

A key challenge is that biodegradable polymers do not behave like conventional plastic materials and therefore require adapted methodologies for characterisation, biodegradation and risk assessment. To address this, the project combines early-stage regulatory review, requirements definition, and targeted data collection for modelling. High-quality experimental information on material properties, toxicity, and environmental degradation will support the development of digital twins capable of predicting biodegradation processes.

The safety assessment integrates new in vitro methods to evaluate potential hazards to human health, including cytotoxicity, genotoxicity, skin irritation and inflammation using relevant human cell models. Environmental safety is addressed through ecotoxicological testing in freshwater, marine and terrestrial systems. Suitability for food and cosmetic contact is evaluated through migration testing and risk assessment based on European legislation.

Sustainability is examined through LCA, S-LCA and LCC, complemented by circularity indicators to capture resource efficiency, end-of-life performance and potential environmental benefits compared to fossil-based packaging.

Overall, this work provides a comprehensive framework for evaluating biodegradable polymers that integrates regulatory compliance, mechanistic understanding of biodegradation, human and environmental safety and holistic sustainability. The results will support the safe implementation of biodegradable packaging and offer transferable approaches for the broader assessment of emerging polymer materials.

Disclaimer/Disclosure:

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3.19.P-We288 Safe and Sustainable by Design Development of Next-Generation Biodegradable Polymers for Circular Packaging Applications

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The transition towards safer, more sustainable packaging materials requires new biodegradable polymers that combine high technical performance with improved end-of-life fate. Despite increasing market demand, most current biodegradable plastics are still limited by suboptimal degradation in real environments, insufficient barrier or mechanical properties, and the lack of harmonised methodologies to assess their safety and sustainability. In this context, BioPackMan aims to design and demonstrate new biodegradable polymer materials for packaging applications by applying a fully integrated Safe and Sustainable by Design (SSbD) framework, supporting the deployment of circular biobased solutions.

The project explores an extended space of biodegradable polymers, including PHAs, PLA, PBS/PBSA and tailored copolymers, combined with sustainable additives such as natural nanocellulose, nanolignin and plant-derived functional ingredients. BioPackMan focuses on the development of at least four innovative biodegradable formulations and multiple additive systems to enhance mechanical performance, barrier properties and controlled biodegradation. Two advanced processing technologies will support the production of both rigid and flexible packaging formats.

A central objective of BioPackMan is to apply SSbD principles from early design stages onwards, integrating hazard assessment, human and environmental safety, exposure considerations and life-cycle sustainability, enabling iterative feedback loops between material development, safety assessment, performance testing and sustainability evaluation. This ensures that the selected formulations and processing routes are aligned with regulatory expectations, minimise potential hazards, optimise resource efficiency and deliver favourable end-of-life scenarios. Packaging demonstrators for food, home-care and personal-care applications will validate industrial relevance and allow benchmarking against conventional fossil-based packaging.

BioPackMan will generate scientific and technical evidence to support the design, assessment and demonstration of safe, sustainable and circular biodegradable polymers. The project will contribute to refining SSbD implementation for emerging biobased materials, provide methodologies for robust biodegradation and safety assessment, and deliver demonstrators and recommendations that facilitate the uptake of next-generation biodegradable packaging solutions in European value chains.

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3.19.P-We289 Safe and Sustainable Bio-based Polymers: Assessing safety and environmental impact of 5-(Hydroxymethyl)furfural Derivates in the CERISEA Project

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The CERISEA project is a pioneering large-scale initiative within the European bio-based chemical sector, dedicated to establishing a sustainable and circular production pathway for 5-Hydroxymethylfurfural (5-HMF). Sourced from non-food biomass side streams, 5-HMF serves as a versatile platform chemical for producing polymers, resins, and crop science solutions. Fully aligned with the European Green Deal, CERISEA's core innovation is its comprehensive application of the Safe and Sustainable by Design (SSbD) framework to systematically assess and mitigate the safety and environmental impacts across the entire value chain, from initial feedstock to final product.

The project's methodology is built on a structured, iterative application of SSbD principles. This begins with a detailed scoping analysis to define system boundaries and characterize the potential environmental fate of HMF and its derivatives. The safety assessment pillar rigorously follows OECD guidelines and REACH standards to proactively identify and mitigate hazards for human health and the environment, ensuring regulatory compliance from the outset. Concurrently, the environmental sustainability is evaluated through Life Cycle Assessment (LCA) to quantify the project's footprint, demonstrating a targeted reduction of 10,200 tons of CO₂ emissions annually compared to conventional fossil-based production. A key aspect of the environmental work involves using advanced predictive and mechanistic models to understand the biodegradation pathways and exposure potential of the resulting bio-based polymers, ensuring they break down safely without leading to persistent accumulation in ecosystems.

By seamlessly integrating cutting-edge production technology with rigorous, tiered SSbD assessments and strong stakeholder engagement, the CERISEA project successfully validates an economically viable and industrially scalable model for bio-based chemical production. It conclusively demonstrates that high-volume manufacturing can be achieved without compromising on safety or sustainability, thereby paving the way for a greener, more resilient, and circular European chemical industry.

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3.19.P-We290 Connecting Disciplines to Shape Sustainable Bioplastics Futures: A System-Dynamics Meta-Framework

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The environmental and societal impacts of plastics necessitate a transition to a sustainable plastic system; however, the complexity and limited understanding of this system hinder effective interventions. Interventions tend to focus on technology or policy, often overlooking broader, interconnected sustainability challenges within plastic systems. There is a need to shift from fragmented approaches to holistic plastic-transition studies. To address this, the Plastic Transition Meta-Framework (Plastic TMF) was developed and presented as a comprehensive framework for analyzing plastic systems and transition dynamics through an interdisciplinary lens. The framework aims to clarify transition complexities and guide future research by integrating sustainability dimensions (people, planet, profit) into an assessment of the system dynamics of the plastic system. Developed through a multi-step process, it builds on a thorough PRISMA-based literature review and integrates existing system and transition dynamics frameworks. The framework incorporates specific components and related indicators to operationalize six interconnected systems: socioeconomic, technological, ecological, driving forces, societal goals, and planned interventions. This structure enables a detailed assessment of system dynamics and clarifies the connections between them. By distinguishing the status quo, system dynamics, and transition dynamics, this framework provides deeper insights into plastic transitions. This interdisciplinary approach addresses key gaps in the existing literature by integrating and advancing diverse perspectives. While its comprehensive nature offers new insights, the framework's applicability may vary depending on specific contexts and practitioners' needs. A complementary case study was conducted to implement the framework. The study explores future development pathways for PHA bioplastics through dynamic material flow analysis, assessing potential technological and legislative interventions and their impacts. All in all, it demonstrates how the framework links current system assessments with future sustainability transitions in plastics. This contribution advances the field by providing a conceptual tool for navigating the complex challenges of plastic sustainability in the future.

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3.20.A PFAS in Terrestrial & Aquatic Ecosystems: Advances in Understanding of Fate and Effects

3.20.A.T-01 Sea Spray Aerosols and other Per- and Polyfluoroalkyl Substances (PFAS) Sources on an Arctic Volcano

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Per- and polyfluoroalkyl substances (PFAS) are a diverse group of organofluorine contaminants found worldwide, including in the remote Arctic. While long-range atmospheric degradation of precursors has become established as a major contributor to PFAS deposition in remote environments, the role of sea spray aerosols (SSAs) remains poorly understood in the field. SSAs, generated by wave action and bubble bursting, may provide an additional significant pathway for PFAS transfer from ocean to atmosphere.

This study investigated PFAS deposition on Jan Mayen, a remote Arctic island in the North Atlantic, to evaluate the potential influence of SSAs alongside other sources. Surface snow samples and surface water samples on the island were collected for this investigation. Ten snow samples were collected inland along a transect (2.5 – 6.8 km from the coast) up the world's northernmost active volcano (2277 m.a.s.l.). Melted snow and water samples were analysed for PFAS, and used to investigate local activities, glacier runoff, atmospheric precursor degradation and SSAs as sources of PFAS in the remote Arctic coastal environment.

Results indicated that local sources were spatially limited (<0.5 km). As such the transect up the volcano represented just processes of the remote Arctic environment. At higher elevations on the transect, PFAS profiles were dominated by trifluoroacetic acid and perfluoropropanoic acid and perfluorobutanoic acid, consistent with atmospheric precursor degradation. Some PFAS concentrations decreased with lower elevation due to increased summer snowmelt, with glacier runoff transferring PFAS to downstream aquatic ecosystems. In contrast, several longer chain perfluoroalkyl acids, including perfluorooctanesulfonic acid and perfluorooctanoic acid, were still elevated in surface snow <500 m.a.s.l., coinciding with elevated sodium ion concentrations, suggesting SSA influence from nearby wave action on the coastline (<4.2 km away). These chain length dependent results confirm existing knowledge that only those most surface active PFAAs are expected to be enriched in SSAs.

These results demonstrated the importance of SSAs as a source of PFAS in coastal areas, whereas the atmospheric degradation of precursors dominated as a PFAS source at locations removed from maritime air mass influence at higher elevations. Understanding SSA as a PFAS source is important for assessing PFAS exposure in coastal ecosystems/agriculture and drinking water sources.

3.20.A.T-02 Environmental and Human Health Risks of Short-Chain PFAS Falling Under the Purview of the Montreal Protocol and Amendments

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Trifluoroacetic acid (TFA) is a breakdown product of several refrigerant gases that fall under the purview of the Montreal Protocol (MP) and amendments. Perfluoro propionic acid (PFPrA) and perfluoro butanoic acid (PFBA) are short-chain PFAS that are used as blowing agents for insulation and also fall under the purview of the MP. TFA is widely used in industry as a precursor for synthesis and a reagent and large quantities are released into the environment. It is also a final residue of some agrochemicals and pharmaceuticals containing the CF₃ group. The -CF₃ group, a pseudohalogen, confers beneficial properties to these chemicals, but they could degrade to produce TFA. These three perfluorinated acids have low pK_a values and accumulate as salts in terminal sinks like the oceans and endorheic lakes with no outflows. They are globally distributed in surface waters. With the possible exception of TFA, they are anthropogenic and are persistent in the environment. Their intensive properties, particularly their solubility in water allows them to be easily excreted by animals and, unlike the longer-chain PFAS, they are not bioaccumulative in food-webs. However, their persistence has raised concerns for potential adverse effects in the environment. This presentation is the culmination of an extensive characterization of environmental fate and effects of these acids in the environment and humans. We have used probabilistic approaches such as species sensitivity distributions combined with cumulative frequency

distributions of environmental concentrations to characterize hazards and risks to humans and the environment. The results of this analysis suggest that predicted amounts to be released in the next 75 years through from the degradation of compounds related to the Montreal Protocol, are of de minimis risk to ecosystem and human health. However, the same is not necessarily true for other sources in the environment.

3.20.A.T-03 Small Creatures, Big Contaminants: PFAS uptake in macroinvertebrates

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Per- and polyfluoroalkyl substances (PFAS) are widely distributed in freshwater ecosystems and can enter the environment through point sources, such as wastewater treatment facilities, or diffuse sources, such as urban runoff. While research has primarily focused on the impact of point-source PFAS, the effect of diffuse sources on biota remains poorly understood; despite urban environments being associated with elevated PFAS. Macroinvertebrates are well-suited to biomonitoring because they live within the water column and occupy key positions in aquatic food webs, providing insight into bioaccumulation across trophic levels. Typical assessments include surface water or sediment samples, which provide a static reading, while macroinvertebrates offer a unique temporal evaluation of contamination. This study quantified PFAS accumulation in freshwater macroinvertebrates from urban and non-urban streams to evaluate diffuse PFAS and characterise pollution profiles across urban and non-urban environments.

Macroinvertebrates were collected from 29 sites in urban and non-urban areas. Concentrations of 25 PFAS congeners were measured and correlated with catchment imperviousness, dominant land use (%), and distance from the largest city to predict PFAS accumulation and congener distribution.

PFAS were detected extensively across all sites, with twenty PFAS congeners detected in 82% of the macroinvertebrate samples. PFOS was the most dominant and was detected at the highest concentrations at 73 ng/g ww. Urban sites exhibited higher median concentrations, although PFOS, PFDoDA, PFTeDA, and ?29PFAS were the only congeners where levels were significantly elevated. PFOS was positively correlated with the percentage of residential and industrial land cover and was negatively correlated with the percentage of non-urban land. The number of PFAS and long-chain congeners had a positive association with the percentage of residential land, while showing a negative association with the percentage of non-urban land.

This study provides the first evidence of PFAS accumulation in macroinvertebrates from Australian freshwater streams, demonstrating that diffuse urban sources can contribute substantially to trophic exposure, even in areas where point sources are limited. These findings advance our understanding of PFAS behaviour and environmental distribution, highlighting the importance of biota in biomonitoring the accumulation of PFAS to guide chemical management strategies.

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3.20.A.T-04 Covering 57 PFASs in One Shot: An Expanded LC-MS/MS Method for Contaminant Tracing Through the Otter Food Web

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With the ongoing phase-out of legacy per- and polyfluoroalkyl substances (PFASs), numerous emerging and precursor compounds have been introduced as replacements. In this study, we developed and validated a sensitive and robust liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method for the simultaneous quantification of 57 PFASs compounds, including legacy substances, precursors, and emerging PFASs such as chloroperfluoropolyether carboxylates (ClPFPECAs) and perfluoroethylcyclohexane sulfonate (PFECBS). The method achieved limits of detection (LOD) as low as 0.01 ng/g, demonstrating its suitability for trace-level analysis in complex biological matrices. It was further applied to several aquatic species within the otter's food web, enabling comprehensive assessment of PFASs distribution, environmental fate, and bioaccumulation in freshwater ecosystems.

3.20.B PFAS in Terrestrial & Aquatic Ecosystems: Advances in Understanding of Fate and Effects

3.20.B.T-01 PFAS in Fire Foam Contaminated Soils – The Importance of Precursors and Leaching for Risk Assessment

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Per- and polyfluoroalkyl substances (PFAS) comprise a chemically diverse group that includes ultrashort chains and high-molecular-weight polymers, posing challenges for environmental risk assessment. Historically, regulations have focused on a limited set of terminal PFAS, overlooking precursor compounds that transform into persistent perfluoroalkyl acids. Aqueous film-forming foams (AFFFs), widely used for fire suppression, are major sources of these precursors, which exhibit strong sorption and some also long-term stability in soils. This study investigates PFAS concentrations, composition and leaching behavior in source-zone soils from Swedish airports to give input to risk assessment strategies. Soils were analyzed using extended target lists, total oxidizable precursor (TOP) assay, and suspect screening to identify both original AFFF constituents and possible degradation products. Batch leaching tests (L/S=10) were performed to evaluate mobility. Results show that fluorotelomer-based precursors, particularly 6:2 FTAB, dominate PFAS profiles, with concentrations up to 8,100 µg/kg dry matter. A number of C5-C8 sulphonamides were also identified but at moderate concentrations. TOP assay confirmed precursor presence and transformation potential, with perfluoroalkyl carboxylate (PFCA) increases up to 12,000 µg/kg. Many of the precursors showed structures with quaternary nitrogen head-groups. Leaching tests revealed substantial mobility, with up to 79% of PFAS content released, and branched forms leaching slightly more than linear forms. Sorption coefficients (Log KOC) indicate significant soil retention for major contaminants as PFOS and 6:2 FTAB, but air-water interface effects may further influence adsorption.

Findings highlight that precursor compounds often exceed regulated PFAS concentrations and significantly contribute to long-term groundwater risk. Incorporating precursors and potential transformation products into analytical and regulatory frameworks is essential for accurate risk assessment and effective remediation planning. The work contributes with insights to guide future contaminated land management and risk evaluation.

3.20.B.T-02 Dynamics of emerging PFAS in a contaminated terrestrial ecosystem

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Previous studies near a fluorochemical plant near Antwerp (Flanders) revealed some of the highest PFAS concentrations ever reported in soils, invertebrates, and songbird eggs. However, these studies mainly focused on a set of legacy PFAS. The main objective was to investigate the occurrence and fate of emerging PFAS identified with non-target analysis in soil and the corresponding accumulation of these compounds in songbird eggs.

Surface soil samples (n=30) were collected in 2024 close to and were pooled into 7 composite samples. Eggs from great tits (*Parus major*) (n=29) and blue tits (*Cyanistes caeruleus*) (n=10) were collected from the same sites in April-May 2022.

Suspect and non-target screening (SS/NTS) analyses were carried out using LC-QTOF-MS. Additionally, ~30 PFAS were analysed by target LC-MS/MS to quantify the most common PFAS, including sulfonates, carboxylates, sulphonamides and their derivatives.

In both soil and eggs, legacy PFAS classes were identified, such as long-chain sulfonates (PFSA), including homologues (up to C16) previously unreported. SS/NTS identified emerging PFAS classes (CL2-3), including pentafluorosulfide-perfluoroalkyl sulfonic acids (SF₅-PFSA), ether-substituted carboxylic acids (PFECAs) and sulfonic acids (PFESA), and keto-PFSA. Certain homologues of SF₅-PFSA (C6, C11), PFECA (C11-C12), and PFESA (C6-C9, C11, C13, C15) were present in eggs, but not in soil, suggesting biotransformation of SF₅-PFSA in birds to shorter homologues.

PFSA were the major PFAS, comprising 24 % of the total PFAS in soil and 86 % in eggs. In both matrices, PFOS was the most predominant (up to 1,030 ng/g in soil and 51,000 and 3,600 ng/g ww in great tit and blue tit eggs, respectively). Yet, the prevalence of FASA (FOSA; 2301 ng/g) and FASAA (Et-FOSAA; 7108 ng/g) was observed in soil. SF₅-PFSA (3900 ng/g; 2% of total PFAS) and keto-PFAS (<1%) were both previously identified in soils impacted by AFFF. In great tit eggs (more contaminated than blue tit eggs), total SF₅-PFSA level reached up to 6200 ng/g, corresponding to 5% of the total PFAS burden. Among SF₅-PFSA, the C9-homologue was the most predominant in both matrices, followed by the C8-homologue.

The comparison of biotic and abiotic matrices showed some overlaps but also differences in contamination profiles and homologue distributions. This emphasizes that SS/NTS approaches have the potential to identify overlooked PFAS to better understand and mitigate adverse effects.

3.20.B.T-03 Linking PFAS Structure to Plant Uptake: Differential Accumulation in Root and Fruit Crops
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Since the early 2000s, restrictions on legacy PFAS such as PFOS and PFOA have driven the development of novel PFAS incorporating shorter chains, ether groups, or halogen substitutions; however, emerging evidence suggests several novel PFAS may equal or exceed their legacy counterparts in persistence and bioaccumulation. As practices such as treated wastewater irrigation and biosolids application expand, understanding how structural modifications influence plant uptake of PFAS is critical. This study aimed to evaluate structural drivers of PFAS bioaccumulation in edible crops and support the development of mechanistic models to inform future agricultural practices. Tomatoes (*S. lycopersicum*) and carrots (*D. carota*) were grown in hydroponic solution containing ten representative PFAS (5 µg/L each). Leaves and edible tissues were sampled every 9 d to monitor PFAS accumulation over time, and whole plants were harvested at maturity for quantification of PFAS in roots, shoots, leaves, and fruits, with corresponding bioaccumulation factors (BAFs) calculated. In tomato roots, 6:2 Cl-PFESA accumulated to the highest extent (1040.8 µg/g), exceeding but followed by PFOS (745.4 µg/g). PFASs accumulated more strongly than PFCAs of equal chain length, and short-chain PFAS showed relatively low root retention, consistent with high mobility. Similar patterns were observed in shoots, with 6:2 Cl-PFESA reaching 889.5 and PFOS 341.4 µg/g. Leaves displayed a distinct distribution, with ADONA accumulating most strongly (5202.9 µg/g in young leaves; 1776.3 µg/g in old leaves), followed by PFOA (2431.4 and 1500.5 µg/g), GenX (1538.8 and 1321.3 µg/g), and PFBA (1503.5 and 1265.9 µg/g). In fruits, accumulation also favored smaller and ether PFAS, with GenX reaching 966.3, PFBA 701.1, PFBS 447.7, and PFHxA 381.9 µg/g. Larger, more hydrophobic PFASs and Cl-substituted PFAS were detected at very low levels in fruits. Calculated BAFs reinforced these trends, with long-chain, Cl-substituted PFAS exhibiting higher BAFs in roots and shoots, while smaller and ether-containing compounds showed enhanced translocation and higher BAFs in leaves and fruits. Ongoing carrot analyses and equation modelling are expected to refine these structure–uptake relationships in a root vegetable system. A better understanding of how specific molecular features help govern plant PFAS bioaccumulation may guide safer chemical design and management strategies to reduce dietary exposures from crop consumption.

Disclaimer/Disclosure:

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3.20.P PFAS in Terrestrial & Aquatic Ecosystems: Advances in Understanding of Fate and Effects

3.20.P-Tu329 Evaluation of the CTC PAL RSi online SPE system for PFAS analysis in environmental water matrices

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants requiring robust analytical workflows for trace-level detection in complex water matrices. This study evaluates the CTC PAL RSi autosampler configured for online solid-phase extraction (SPE) coupled to a Waters Xevo™ TQ-Sµ triple quadrupole mass spectrometer for the analysis of PFAS. Included are the list of 48 PFAS in UK Drinking Water Inspectorate (DWI) including the 20 PFAS contained in the 2020 EU Drinking Water Directive (EU 2020/2184) and emerging zwitterionic/cationic species associated with aqueous film-forming foams (AFFFs) totalling 55 analytes.

To address zwitterionic and cationic PFAS species, the method employs rapid polarity switching during MRM acquisition, leveraging the TQ-Sµ's fast polarity switching capability (15 ms) to ensure accurate detection across diverse PFAS chemistries.

Using Oasis™ HLB SPE chemistry in a single-column setup, the system demonstrated suitability for quantitative analysis of most target PFAS across drinking water, groundwater, river water, and surface water matrices, with limitations observed for PFBA and neutral PFAS. The workflow operates in trap-and-elute mode,

enabling large-volume injections (up to 5000 µL) and sequential sample processing.

Key considerations include minimizing carryover through solvent blanks and maintaining low system background via PFAS-free components and rigorous cleaning protocols. The platform offers flexibility for multiple sample introduction techniques and enhanced contamination control, making it well-suited for high-throughput PFAS monitoring in relatively clean water samples.

3.20.P-Tu330 Sustained Accuracy in PFAS Analysis: Robust Instrument Performance for Environmental Monitoring

David Gould¹, Aron Dekkers¹ and Peter Hancock², (1)Waters Corporation, United Kingdom, (2)Waters Corporation, Germany

Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants of growing regulatory concern, requiring accurate identification, quantification, and confirmation across diverse environmental matrices. In a robustness study exceeding 17,500 injections, the Xevo™ TQ Absolute XR Mass Spectrometer was evaluated for long-term performance using landfill leachate, biosolids, and river water samples.

Eleven PFAS residues were consistently detected in leachate extracts, including PFBA and PFPeA - compounds traditionally considered challenging to confirm due to limited product ion availability. Sensitive detection of the m/z 19 product ion enabled reliable confirmation within standard multiple reaction monitoring (MRM) acquisition, supported by enhanced low-mass transmission in waters_connect™ for Quantitation Software. The Totals feature in waters_connect facilitated streamlined reporting of branched, linear, and total concentrations for PFHxS and PFOS, ensuring compliance with evolving regional requirements.

Over 120 days, the system demonstrated exceptional robustness, consistent quantitation, and minimized unscheduled maintenance, highlighting its suitability for high-confidence PFAS analysis without secondary confirmation methods. This capability supports laboratories in meeting stringent and rapidly changing regulatory frameworks, reducing the need for additional confirmatory techniques and accelerating compliance reporting.

3.20.P-Tu331 Simulation-based Evaluation of Long-term Trends in Environmental PFOS and PFOA Concentrations Across Japan Using the Multimedia Fate Model (G-CIEMS)

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Many studies have been conducted to estimate environmental levels of per- and polyfluoroalkyl substances (PFAS), a group comprising more than 10,000 compounds. This issue originated with the production of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) for applications such as textiles and packaging materials since the 1950s. PFOS was listed under Annex B (Restriction) of the Stockholm Convention in 2009, and PFOA under Annex A (Elimination) in 2019. In Japan, a maximum limit of 50 ng/L for the combined concentration of PFOS and PFOA in drinking water was established in June 2025, and the same limit will be applied as an environmental quality standard for water pollution from April 2026. To estimate PFOS and PFOA concentrations in rivers and their temporal trends, several monitoring campaigns have been conducted in Japan. However, distinguishing sampling sites influenced by upstream point sources from those affected by “ordinary sources,” such as households and general human activities, remains challenging. This study provides a comprehensive evaluation of baseline PFOS and PFOA concentrations in Japan’s environment over the past five decades using the multimedia fate model G-CIEMS. To construct long-term emission data, we analyzed multiple monitoring datasets useful for estimating representative temporal trends of PFOS and PFOA emissions, including sediment core samples and flux estimations based on observed concentrations. By integrating historical monitoring data and sediment core analyses, we reconstructed emission trends and simulated their long-term environmental fate. Finally, by comparing simulated concentrations with observed data in the atmosphere, river water, and river sediments, we assessed the reliability of the emission dataset and the simulated long-term trends across Japan.

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3.20.P-Tu332 Environmental Fate and Effects of Low Molecular Weight Perfluoropolyether (PFPE) fluids: a Weight of Evidence approach

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Perfluoropolyethers (PFPEs) are a family of liquid perfluoropolymers characterized by a carbon backbone with repeating ether units, in which all hydrogen atoms are replaced by fluorine. Low molecular weight (MW) PFPEs exist as low-viscosity fluids and, due to their combination of dielectric and mechanical properties, are essential materials for heat transfer and testing applications in the semiconductor industry.

In this work, new data and existing information were combined using a WoE approach to assess the environmental fate of a specific family of low MW PFPEs, commercially known as Galden®.

The assessment covers three Galden® grades with the same structure but different MWs (410, 610, 870 Da), allowing trend evaluation within the product family. Data include measured physicochemical properties for each grade (in order of increasing MW: vapor pressure 14,800, 560, 15 Pa; water solubility 0.5, 1.0, 2.1 ppm; LogKow 5.6, 2.3, 2.4). LogKoc was calculated for all grades (4.6, 2.0, 2.0), while LogKoa was measured for the lowest MW (0.9) and calculated for the others (0.2, 1.9). The dataset also includes experimental partitioning tests (sediment, water, air), environmental distribution modeling, and results of standard ecotoxicological tests, all showing no signs of acute toxicity.

All evidence consistently shows that environmental distribution is strongly dominated by volatility, with the atmosphere being the primary compartment for all three products (? 99.9%). Partitioning into other compartments (water, sediments, soil) increases slightly with increasing MW, which corresponds to a decrease in volatility; however, distribution in these compartments remains very limited (< 0.1%). Distribution in biota is predicted to be negligible. Although Kow alone, as an indicator of bioaccumulation in aquatic life, might suggest that the grade with the lowest MW has potential for bioaccumulation, this potential is neutralised by the substance's high volatility, which prevents exposure of aquatic organisms. For the same reason, aquatic toxicity in natural systems is not considered critical.

This outcome highlights the importance of considering multiple pieces of evidence when assessing the environmental fate of chemicals, especially for volatile substances. It also demonstrates that, depending on its environmental distribution, some hazard properties may be of limited significance for the overall hazard assessment of a chemical.

Disclaimer/Disclosure:

All authors are employed at Syensqo.

3.20.P-Tu333 PFAS enrichment in the surface microlayer and lake foam: a multi-lake study in the Finger Lakes of NY

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Per- and polyfluoroalkyl substances (PFAS) are surfactants that can accumulate at the air-water interface, resulting in elevated PFAS levels at the water's surface. In recent years, large quantities of surface foam have been observed on many of the Finger Lakes in the United States. The 11 Finger lakes provide drinking water to over 2 million people and are home to world-renowned wineries. Because foam forms from water at the surface microlayer (SML, top 1mm), both foam and the SML might contain elevated PFAS levels compared to water below. Preferential partitioning of PFAS into foam and the SML could pose higher risks for wildlife and humans that frequently interact with the water's surface. The foam also accumulates along shorelines, exposing lakeshore properties. We surveyed 40 PFAS compounds and their concentrations in lake foam, the SML, and water column of five of the larger, lower-nutrient (oligotrophic to mesotrophic) Finger Lakes. Our results show total PFAS enrichment in foam relative to the underlying water, with some PFAS compounds detectable only in foam. PFAS in the SML are variable, and likely dependent on the formation of surface slicks. Our results advance the understanding of PFAS fate and transport in aquatic systems, as well as how different PFAS compounds partition and accumulate in foams. In collaboration with lake associations, we also developed and distributed a web-based foam reporting platform. Other watersheds can easily adapt the reporting tool to increase documentation of surface foams in natural waters.

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3.20.P-Tu334 Tracing PFAS in Lake Como Basin: from WWTPs to Rivers and The Lake

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Per- and polyfluoroalkyl substances (PFAS) are persistent and mobile contaminants. Owing to their extensive use and persistent nature, PFAS are now widespread into aquatic environments, ending directly by PFAS containing coatings (e.g., paper and textile coatings) and indirectly coming from leachate from landfills and effluent of wastewater treatment plants (WWTP). The persistence and high mobility of PFAS in water bodies, along with their potential to bioaccumulate in aquatic organisms, have made these ecosystems a major focus of scientific research, especially considering the crucial ecological role that surface water play. This study provides a comprehensive assessment of PFAS contamination in surface waters of the Como basin, with a focus on the effects rainfall events. Lake Como, due to its morphological characteristics and slow water renewal rate, is a sensitive environment. Since it receives inputs from two WWTPs through minor tributaries, it is potentially vulnerable to chemical pollution.

Overall, around 20 PFAS compounds were found in both riverine and lacustrine environments under normal and overflow conditions. Results highlight the changing profile from river samples before and after the Como WWTP, and then in lake samples. Lake water sampled in littoral and pelagic areas in epilimnion and also in hypolimnion (-40m) showed a slightly different chemical fingerprint, likely influenced by dilution from water coming from northern areas of the lake, and other processes.

3.20.P-Tu335 Assessing Multiple PFAS Collectively: Insights from a Scenario-Based Analysis using Multimedia Exposure Model

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Growing scientific and regulatory attention has highlighted the urgent need to better characterize the environmental and human-health implications of per- and polyfluoroalkyl substances (PFASs). To prioritize PFASs for future risk management, exposure assessment and risk assessment based on realistic environmental scenarios are essential. Under thousands of PFAS compounds said to exist, rapid assessment requires effective analytical modelling alongside advances in monitoring and analytical techniques. In this study, we aimed to verify whether significant differences among PFAS compounds could be identified through a consistent analytical procedure and attempted to visualize these differences to explore the possibility of grouping structurally or behaviorally similar compounds. We also employed a well-developed multimedia model to identify exposure pathways for expert selected multiple PFAS under a specific use scenario. Our results revealed both the effects of individual substances and the effects of selecting functional use categories, demonstrating the complexity of simultaneously analyzing multiple types of PFASs. Moreover, our results suggested that classification based on exposure characteristics is feasible; for instance, the atmospheric distribution-dominant substance group exhibited significantly lower total exposure levels than the soil and water distribution-dominant substance groups. Our findings highlighted the need of precariousness in regulating multiple PFAS collectively and demonstrated the necessity for individual exposure assessments and comparative evaluations. In our presentation, we will discuss the advantages of model-based PFAS exposure assessment, along with scenarios and substances selected, and outline future directions for advancing PFAS evaluation.

3.20.P-Tu336 Flushed Through the System: A Sub-Kilometer Investigation of PFAS Pathways from Urban Rivers to Coastal Waters

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Per- and polyfluoroalkyl substances (PFAS) are a large group of man-made chemicals, defined by the Organisation for Economic Co-operation and Development (OECD) as any compound with a (-CF₃) or (-CF₂-) moiety. This strong carbon-fluorine bond renders these compounds resistant to degradation and impacts

hydrophobic and oleophobic properties.

These physico-chemical properties are highly attractive to industry, with PFAS being utilised in a range of industrial sectors including chemical manufacturing, electronics, pharmaceuticals, and firefighting foams as well as coatings on consumer goods. However, these same properties contribute to the persistence of PFAS in the environment. Widespread use and resistance to degradation have resulted in PFAS becoming ubiquitous in the natural environment. Even in areas without fluorochemical production, such as Ireland, PFAS can enter the environment through industrial and municipal wastewater, firefighting activities, and urban runoff, often travelling through stormwater networks before being discharged to surface waters.

This study aimed to explore the influence of stormwater outfalls on the concentration of 33 PFAS in the Liffey Estuary, Dublin, Ireland. Sub-kilometer sampling was conducted at individual outfalls along the river to assess variability in PFAS loadings.

Water samples were extracted by solid phase extraction using Oasis WAX cartridges, and analysed by high-pressure liquid chromatography (Sciex Exion) coupled with time-of-flight mass spectrometry (Sciex 5600+ triple TOF-MS).

Twenty-four PFAS were detected across the estuary, including long-chain compounds and their short-chain replacements. Total PFAS concentrations ranged from 6.20 to 91.48 ng/L, with highest total concentrations downstream of waste treatment facilities. Spatial variation in PFAS class profiles was evident across the upper and lower estuary, with the number of PFAS detected ranging from 2 to 14 compounds. Detections and concentrations varied markedly between outfalls within a few hundred meters of each other. Multivariate analysis indicated that stormwater outfalls act as a diffuse source of PFAS in the Liffey Estuary, along with unique PFAS profiles being attributed to localised inputs such as waste facilities and marina activities.

These findings highlight the need for high-resolution spatial monitoring to improve PFAS source attribution and understanding of mobility pathways in urban surface waters.

3.20.P-Tu337 PFAS Partitioning in the Mississippi River

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The present study uses publicly available PFAS data collected from surface water, pore water, sediments, surface microlayers, and fish tissue in Pool 2 of the Mississippi River to estimate partitioning coefficients across a broad range of PFAS, including FASAs and ultrashort-chain PFAS, some of which have limited fate and transport data reported in freshwater systems. Sorption coefficients (K_d and K_{oc}), microlayer enrichment factors (EF), and bioaccumulation factors (BAF) reported here can inform considerations for environmental fate and transport modelling, risk assessment, and treatment technologies.

This study reports on field observations of partitioning between surface water, sediment, pore water, surface microlayer, and fish tissue across 46 measured PFAS. The data includes 56 locations sampled for PFAS in surface water, pore water, sediment, and surface microlayer and 865 fish samples during the summer of 2021.

PFAS with detections in at least 80% of samples for at least one media are PFBA, PFBS, PFHxA, PFHxS, PFOA, PFOS, PFPeA and TFA. PFAS with detections in at least 50% of samples in at least one media are TFSI, N-EtFOSAA, PFHpA, PFPA, and TFMS. TFA was the most abundant PFAS in surface water samples by about one order of magnitude, with values detected in the single-digit parts per billion. Other short-chain and ultra-short chain PFAS, including TFMS, TFSI, PFBA, also dominated surface water samples. Sediments and pore water more reflected impact from legacy PFAS such as PFOA and PFOS.

K_d values increase with increasing chain length for both PFCAs and PFSAAs, especially for compounds with more than 6 carbons. There was limited difference between the two classes, except for PFOS, which has partitioning coefficients 0.5 to 1.0-log higher than those for PFOA. BAFs observed in this study were similar to other studies for PFAAs, and reflect an early report of BAFs in ultra-short TFA, TFMS, and TFSI, all with values similar to those for PFBA. Partitioning from surface water to microlayer is limited to a factor of 1 to 10 for most PFAS, with more partitioning to microlayer for long-chain PFAAs over short-chain PFAAs. The FASAs detected in microlayer samples, N-EtFOSA, N-EtFOSAA, and PFOSA, all have much higher microlayer concentration factors than other PFAS, around 1,000x (3-log), more similar to sediment K_d values than other microlayer EFs.

3.20.P-Tu338 A 25-year study of the presence of PFAS in wild little penguins (*Eudyptula minor*) from around Sydney, Australia

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Per- and polyfluoroalkyl substances (PFAS) are persistent organic pollutants designed to be highly stable, making them widespread and enduring in the environment. Their presence in water bodies is concerning because they bioaccumulate, yet few studies have examined their occurrence in Australian aquatic organisms. To assess long-term PFAS exposure in a native Australian species residing off the east-coast of Australia, little penguins (*Eudyptula minor*) were chosen as indicator species. Little penguins are the only penguin species to breed on the mainland in the state of New South Wales (NSW), where only one mainland colony is located at Manly Cove in Sydney Harbor National Park. This colony is classified as endangered due to threats from predators, urban development, and human disturbance. More broadly, little penguins across NSW are also in decline. The short lifespan and strong site fidelity of the species make them ideal for studying temporal changes.

The presence of 71 PFAS was investigated in little penguin livers using a rapid sonication-based extraction followed by solid phase extraction for purification. Analysis was performed via liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS, Qtrap). The study analysed 93 wet liver samples, as part of an opportunistic collection and storage program spanning 25 years (1998–2023). These samples were obtained from wild penguins found across Sydney area and other regions of NSW. Age, sex, collection location, and cause of death including apparently healthy individuals and ill penguins was recorded for each sample.

42 PFAS were detected at concentrations ranging from 41 ng/g to 3130 ng/g (total PFAS). PFOS was present in all samples, consistently alongside at least one other PFAS, and was the most abundant compound detected in the penguins. PFHxS, 6:2 FTS, PFHpS and PFDODA were found in more than 50% of samples. Temporal variations were observed over the 25-year period for five PFAS compounds, including PFOS, with a decline in PFOS concentrations between 2009 and 2023. Male penguins exhibited statistically higher total PFAS concentration levels than females. No significant differences were detected between juveniles and adults. Although PFAS concentrations in liver tissues have declined over time, the little penguin population remains exposed to appreciable levels, highlighting the need for further research and continued protective measures for the colony, and the species more broadly.

Disclaimer/Disclosure:

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3.20.P-Tu339 Evaluation of PFAS in Higher Trophic Level Bird Eggs

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Perfluoroalkyl substances (PFAS) are widespread in aquatic food webs, yet information on PFAS in bird eggs remains limited for most species. Eggs of piscivorous birds provide an integrated measure of exposure during pre-laying foraging and can offer insight into background levels and potential ecological risk. The objective of this study was to characterize PFAS in eggs of three piscivorous bird species and to evaluate how measured concentrations compare with published toxicity thresholds for perfluorooctane sulfonate (PFOS).

Eggs of common loon, osprey, and double-crested cormorant collected from sites in Maine and New Hampshire were analyzed for 40 PFAS using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Samples represented multiple nesting periods and were derived from homogenized archived eggs. Spatial differences were observed within species, with common loon eggs in Maine showing significant variation among locations. PFOS was the dominant compound in all species. Trends over time were assessed for

Maine common loon eggs, showing declining concentrations of 7:3 fluorotelomer carboxylic acid and variable patterns for several long-chain acids. Osprey eggs exhibited declines for multiple PFAS, although site coverage limited interpretation.

Background concentrations were estimated using distributional approaches and indicated higher PFOS levels in New Hampshire relative to Maine. When compared with available PFOS toxicity thresholds for bird eggs, a portion of samples from both states exceeded the lowest published value, while none approached higher thresholds. Population data available for common loons suggest that the lowest threshold may be conservative relative to observed field conditions.

Overall, PFAS levels were greatest near denser human activity and closer to known sources. This work highlights the need for additional field-based studies to determine what dietary exposure levels and egg concentrations may indicate risk for piscivorous bird populations. Findings support improved interpretation of biomonitoring data and provide initial background values for species with limited prior reporting.

3.20.P-Tu340 Integrated monitoring and toxicological assessment of PFAS in aquatic ecosystems: Human exposure risks in UK and Spanish populations and insights from cellular bioenergetic profiling

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Per- and polyfluoroalkyl substances (PFAS) are widespread contaminants characterised by extreme persistence, environmental mobility and documented biological effects. Their presence in aquatic environments raises concerns for ecosystem integrity and human exposure through fish consumption. This study combines large-scale monitoring with mechanistic in vitro assays to assess PFAS occurrence, toxicological behaviour and potential exposure risks in the United Kingdom and Spain. Between 2020 and 2024, more than 10,000 surface water, groundwater and saline water samples, together with 288 edible fish from 16 species, were analysed for 47 PFAS using targeted LC-MS/MS and LC-HRMS suspect screening. PFAS were frequently detected in all matrices. Spanish fish showed elevated levels of long-chain PFAS and emerging PFAS such as PFNA and 6:2 FTS, whereas UK fish were dominated by PFOS. In contrast, UK waters displayed higher proportions of PFOA and short-chain PFAS (e.g., PFPeA, PFHxA), consistent with their mobility and ongoing inputs. Estimated dietary exposure via fish exceeded the EFSA TWI (4.4 ng/kg bw/week) by more than five-fold in Spain, indicating concern for frequent consumers. Spatial analysis revealed a hotspot in Moreton-in-Marsh (UK), where high PFAS in freshwater suggest localised sources requiring investigation. To evaluate mechanistic toxicity, zebrafish fibroblast (ZF4) cells were exposed to 19 PFAS (10–1000 µM), and bioenergetic function was assessed using Seahorse XF analysis and CCK-8 viability assays. PFAS exposure produced clear, structure-dependent effects: mid- and long-chain PFAS reduced maximal respiration and spare capacity while increasing glycolysis, indicating a compensatory shift in energy metabolism. Long-chain PFAS caused the strongest decreases in viability and mitochondrial performance, aligning with known structure-activity trends. These findings support the use of bioenergetic assays as New Approach Methodologies (NAMs) for mechanistic PFAS hazard characterisation. Overall, this assessment highlights significant PFAS contamination in aquatic environments, dietary exposure risks, the presence of localised hotspots and mechanistic evidence of mitochondrial disruption. Together, the results strengthen the basis for integrated ecological and human-health risk assessment and emphasise the need for targeted actions to mitigate PFAS contamination in freshwater and marine systems.

Disclaimer/Disclosure:

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3.20.P-Tu341 Toxicological Assessment of PFOA and PFOS in a Bottlenose Dolphin Fibroblast Cell Line

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Due to their persistence and bioaccumulative behaviour, Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonic acid (PFOS) pose significant risks to marine species. Bottlenose dolphin (*Tursiops truncatus*), as apex predator that inhabits coastal regions and frequently interacts with human activities, is particularly susceptible to the accumulation of these pollutants. Consequently, the development of assays for accurately assessing the biological effects of these anthropogenic chemicals in this species appears essential.

This study investigated the toxicological responses of a bottlenose dolphin fibroblast cell line to PFOA and PFOS. Initial cytotoxicity tests, conducted using a luminescent cell viability protocol, assessed half-maximal inhibitory concentrations (IC₅₀) following 24, 48, and 72 hours of exposure to increasing concentrations of each compound. Subsequent experiments examined early apoptosis and necrosis (Annexin V Apoptosis and Necrosis Assay), late apoptosis (Caspase 3/7 Assay), and oxidative stress (Glutathione Assay) at three sub-cytotoxic concentrations (? IC₂₅).

PFOS cytotoxicity was higher than that of PFOA, showing IC₅₀ values around 230 µM at all the examined time points, indicating that the compound's potency is stable over time. Conversely, PFOA IC₅₀ at 24, 48, and 72 hours of exposure resulted in 934, 837.5 and 695.2 µM, respectively. While necrosis was not evident, early apoptosis and oxidative stress were appreciated at concentrations well below the IC₅₀, (i.e. 100 and 300 µM for PFOS and PFOA, respectively). This evidence demonstrated that, although the observed IC₅₀ were quite high (but almost comparable to those reported for these compounds in human cells), both PFAS might elicit subtle effects preceding overt cytotoxicity in this cell model.

This study demonstrated that cetacean in vitro systems represent a valid tool for studying the cellular effects of contaminants of environmental concern (CECs) and advancing understanding of pollutant impacts on marine mammals and ecosystem health.

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3.20.P-Tu342 Influence of DOC and Microplastics on PFAS Bioavailability and Ecophysiological Effects on Zebrafish (*Danio rerio*) Embryos

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Over the last two decades, per- and polyfluoroalkyl substances (PFAS) have gained increased focus as more and more evidence of their biological effects in different biota has been demonstrated; examples in fish include malformations and behavioural aberrations. Globally, the contamination with this class of persistent organic pollutants (POPs) is particularly high in the aquatic environment due to their longevity. Moreover, little is known about their effects in presence of environmental vectors, which have been shown to change bioavailability of POPs by adsorption.

Therefore, the goal of this study is to strengthen knowledge on how climate change and the increasing presence of vectors affect the toxicity of PFAS on the aquatic environment. To this purpose, the effect of four PFAS (PFOA, PFUndA, PFHpA, PFHxDA) were studied in zebrafish embryos, both alone and in combination with one of two vectors. The chosen vectors were dissolved organic carbon (DOC) and microplastics. DOC is a major factor of water brownification, which affects aquatic ecosystems globally. A commercially available fertiliser was used as a DOC proxy due to its high content of humic and fulvic acids. Polyethylene was chosen for the microplastic exposure, as it plays a significant role in single use plastics and can be found in large amounts in the aquatic environment.

To evaluate potential interactions between pollutant and vector, the acute toxicity of PFAS was tested singularly and together with either vector to establish the EC₅₀. In a second step, embryos were exposed to sublethal concentrations until 120hpf and subsequently used to evaluate their thermal tolerance as well as several biomarker assays to measure oxidative stress, dioxin-like activity and metabolic stress. Preliminary results show that the addition of DOC leads to higher acute toxicity, as well as a decrease in the average thermal maximum of zebrafish embryos, suggesting that brownification might increase PFAS toxicity in aquatic organisms and weaken their response to environmental changes.

Disclaimer/Disclosure:

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3.20.P-Tu343 Assessing the Toxicity of PFAS to *Enchytraeus crypticus* – Can Compound Structure be Used to Classify Relative Toxicity?

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Per- and poly-fluoroalkyl substances (PFAS) are contaminants across ecosystems with multiple known release pathways into terrestrial systems, yet the understanding of risks posed to terrestrial organisms is lacking. The vast number of PFAS compounds requires development of approaches to aid in classifying the relative hazard posed across this growing group of contaminants. This study will establish a ranking of lethal concentration data for selected PFAS compounds and form the basis of future work to understand the potential correlations between the structure, chemical properties and toxicological effects of PFAS. We consider the survival effects in the annelid *Enchytraeus crypticus*, a model soil organism, using an acute aquatic toxicity set up to allow survival through time to be monitored over 96 h. The selection of PFAS used were chosen based on evidence of use and environmental presence whilst ensuring there was representatives from four classes with differing functional groups in their structure: perfluorocarboxylic acids, perfluorosulphonic acids, perfluorosulfonamides and fluorotelomers, and within these classes there was at least one short chain and one long chain PFAS selected. Preliminary data support the hypothesis that lethal concentrations will vary depending on the structure of the PFAS, with variation in toxic response seen between the classes of PFAS. Subsequent analysis of internal organism PFAS concentration, from the end of exposures, will assess the uptake of PFAS and determine any toxicokinetic variation. Further work will develop on this to understand toxicodynamics, by assessing PFAS interactions within the organisms during exposure.

3.20.P-Tu344 Multigeneration toxicity and accumulated adverse effects of perfluoro(4-methyl-3,6-dioxaoctane) sulfonate in *daphnia magna*.

GISU PARK, Shinhye Park and Youngsam Kim, KIST-EUROPE, Germany

Structural alternatives of per- and polyfluoroalkyl substances (PFASs) have been widely used in industry, resulting in their ubiquitously distributed presence in the environment and biological system. Among these alternatives, perfluoroalkyl ether sulfonic acid (PFESAs) emerged as next-generation PFAS replacements and increasingly detected in aquatic environments and consumer products. While assumed to be a safer alternative, potassium perfluoro(4-methyl-3,6-dioxaoctane) sulfonate (PFMDS) may pose delayed and generationally compounding risks in aquatic ecosystems. PFMDS shares the specific 'ether-sulfonate' structural motif with 6:2 Cl-PFESA (F-53B), a potent toxicant known for its high bioaccumulation and endocrine-disrupting potential. With growing concerns over ecotoxicological effects, this study presents the comprehensive evidence that PFMDS exhibits transgenerational toxicity amplification. We evaluated toxicity of PFMDS on the keystone model organism *Daphnia magna*, integrating multigenerational and acute with physiological endpoints with transcriptional profiling. Acute exposure yielded a 48-h EC50 of 22.25 mg/L and induced a significant elevation in reactive oxygen species (ROS) levels in adults after 24 hours of exposure. In the chronic test, the first generation (F0) exposed to concentrations up to 10.0 mg/L showed no significant changes in reproduction or growth. However, the second generation (F1) exhibited remarkable physiological impairment at the same concentration. Consequently, reproductive output decreased by over 20%, and significant growth retardation was also observed in the third generation. This pattern of multigenerational toxicity accumulation indicates that adverse effects of PFMDS becomes more pronounced across generation. In understanding of further mechanisms of toxicity, transcriptional analysis was conducted on both chronically and acutely exposed adults and neonates. The expression levels of antioxidant-related gene (SOD, CAT, GST, Hr3) and ecdysteroidogenesis-related gene (Vtg1/2, EcR-A/B, JHE) were examined in chronic exposure, JHE and Vtg expression levels were significantly downregulated. Similarly, in both 24-hour exposed adults and neonates, Vtg expression was also significantly downregulated. Collectively, these findings provide a comprehensive assessment of PFMDS toxicity and demonstrate its amplified adverse effects across generations in *D. magna*, emphasizing important concerns about the long-term ecological safety.

3.20.P-Tu345 Early-Life Transcriptomic Disruptions in Zebrafish Exposed to GenX, NBP2, PFOS, and their Mixture Reveal Chemical- and Dose-Specific Neurodevelopmental toxicity

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Per- and polyfluoroalkyl substances (PFAS) and their emerging alternatives present growing environmental and human health concerns. *Danio rerio* embryos exposed to PFOS, NBP2, GenX, and their mixture (0.1–30 µg/L) did not show morphological deformities upon static exposure. However, biochemical assays revealed a significant increase in reactive oxygen species (ROS), antioxidant enzymes, and acetylcholinesterase (AChE) activity, indicating cellular stress. To understand the mechanistic pathways underlying these responses, *D. rerio* larvae were exposed from 0 to 96 hpf to PFOS, NBP2, GenX, and their binary mixture at concentrations of 0.3 and 30 µg/L and subjected to transcriptome profiling (fold change ≥ 1.5 ; FDR < 0.05). PFOS induced a dose-dependent transcriptional effect aligned with biochemical outcomes. Low-dose PFOS (0.3 µg/L) disrupted synaptic vesicle fusion and glutamatergic/GABAergic transport (*cplx2b/3a/4a*, *syt11b*, *slc17a6a/7a*), while high-dose PFOS (30 µg/L) enhanced dopaminergic GPCR signalling, glutamate biosynthesis, and GABAergic regulation (*drd4a*, *gnal*, *gls2a*, *npas4a*), consistent with elevated ROS and AChE activity. NBP2 at 30 µg/L altered CNS neuron differentiation (*wnt8b*, *nr4a2b*, *lhx6a*) and auditory visual sensory pathways (*trpa1b*, *pcdh15a*, *exorh*, *tmc1*), supporting its neurotoxic potential. GenX showed distinct dose responses: 0.3 µg/L GenX enriched glutamatergic synaptic transmission (*gria2a/3/3b/4a*, *slc17a6a/b*, *grin2bb*), whereas 30 µg/L activated enzyme-linked receptor signalling and oxidative stress pathways, mirroring elevated antioxidant enzyme activities. The mixture exposure elicited the broadest disruptions. At 3 µg/L, it altered mechanosensory behaviour and sensory system development (*scn8aa*, *pkd2l1*, *cib2*). At 30 µg/L, strong endocrine and hormone-responsive pathways (*esr1/2b*, *thrb*, *fgf19/23*, *nr4a2a/b*, *stat5b/6*) were activated, aligning with severe oxidative stress markers. qPCR and behavioural assays (locomotor activity and startle response) corroborated the transcriptomic changes, revealing neurodevelopmental impairments induced by legacy and alternate PFAS. The multi-tiered biochemical, transcriptomic, qPCR, and behavioural analysis demonstrates PFOS, NBP2, GenX, and their mixtures induce oxidative stress-mediated neurodevelopmental toxicity in zebrafish. These findings emphasise the urgent need to integrate molecular endpoints and mixture toxicity considerations into ecological risk assessment for emerging PFAS.

3.20.P-Tu346 From Farm to Table: Field and Laboratory Insights into Bioconcentration, Uptake Kinetics, and Consumer Risk of Per- and Polyfluoroalkyl Substances in Kelp

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Macroalgae such as the native New England kelp, *Saccharina latissima*, represent the fastest-growing sector of aquaculture, providing an increasingly important source of income as climate change impacts traditional fisheries. Because aquaculture farms are predominantly located in estuarine and coastal waters, understanding the influence of environmental contaminants on cultivated kelp is essential. Although previous studies have demonstrated bioaccumulation of heavy metals and legacy pollutants such as Polychlorinated biphenyls (PCBs) in kelp, data is limited for the uptake of emerging contaminants such as per- and polyfluoroalkyl substances (PFAS). PFAS are ubiquitous, environmentally persistent chemicals used in firefighting foams and stain-, water-, and grease-resistant coatings, and are associated with a range of adverse health effects. This study quantifies the bioconcentration potential of *S. latissima* and assesses potential harm to consumer by establishing field-based bioaccumulation factors (BAFs), laboratory-derived bioconcentration factors (BCFs), characterizing kinetic uptake rates for an 11-compound mixture of perfluoroalkyl acids (PFAAs), an anionic class of PFAS, and deriving estimated daily intakes and hazard ratios for different demographics based commercially available seaweed products. Determining BAFs, BCFs and uptake kinetics for *S. latissima* will support evaluations of kelp's potential role as a sink for PFAS in the coastal environment and inform monitoring programs for aquaculture operations to assess potential exposure risks to crops. Additionally, we contextualize the contamination in Rhode Island-sourced kelp to that of other seaweeds available in the global market and provide actionable data to inform risk communication for the consumptions of seaweeds.

3.21 Pharmaceuticals in the Environment – Innovations in Risk Assessment, Regulation, and the Science Globally

3.21.T-01 Connecting the Dots in Terrestrial Pharmaceutical Fate: Towards a Prioritisation Framework

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Pharmaceuticals are now widespread contaminants, yet their behaviour in terrestrial systems remains poorly characterised compared to aquatic environments. The heterogeneity of soils, and resulting variation in physical and chemical properties, complicates predictions of pharmaceutical fate, transport and bioavailability. Continued use of wastewater sludge and reclaimed water in agricultural practises, alongside regulatory shifts that place greater emphasis on chemical persistence and mobility, highlight the urgent need for approaches that can fill data gaps and support prioritisation of high-risk substances.

The framework presented combines data from sludge sorption studies, soil mobility modelling, earthworm uptake experiments, and spatial evaluation of risk in Europe. Experimental work produced 178 linear sorption coefficients for 48 pharmaceuticals across four sludge types, covering a range from 1.3 to 21,846 L/kg. These values showed up to four orders of magnitude variation between compounds and up to two orders of magnitude variation across sludge matrices for individual substances. A machine learning soil mobility model was developed and optimised using random forest algorithms, trained on existing soil sorption data, to predict organic carbon normalised sorption coefficients for more than 1500 human and veterinary pharmaceuticals across seven OECD 106 representative soils. The model performed strongly during cross validation ($R^2 = 0.93$) and external testing (Literature $R^2 = 0.58$, Industry $R^2 = 0.29$) and allowed mobility classification under new European regulatory criteria, with more than half of all assessed substances predicted to be mobile or very mobile. A QuEChERS based method for 41 pharmaceuticals in earthworm tissues and soils achieved recoveries between 50 and 150 percent and supported a reduced scale OECD 317 uptake study design. This generated kinetic soil bioaccumulation factors and porewater bioconcentration factors across five soils, supporting the assessment of pH and organic carbon effects on bioavailability in *Eisenia fetida*.

Predicted mobility outputs were combined with European soil and land use data, and pharmaceutical consumption patterns to map spatial variation in terrestrial exposure, identifying regions with elevated mobility and potential for groundwater contamination.

Together, these components form a practical and scalable framework for assessing and prioritising pharmaceutical exposure and risk in terrestrial systems.

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3.21.T-02 Advancing Drug Emission Modelling Through Systematic Derivation of Human Drug Metabolism and Excretion Data from Regulatory Sources and Mass Balance Study Publications

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Background: Pharmaceutical pollution is a growing concern, with potential risks to both human health and aquatic ecosystems. With the growing diversity of drugs, empirical biomonitoring is becoming increasingly challenging. Hence, emission prediction methodologies based on drug utilization data are gaining importance. A key parameter across many of these models is the so-called excretion fraction (f_{ex}). This factor aims to correct for the fact that many administered drugs, commonly referred to as parent drugs, are not always excreted (entirely) in their unchanged form but rather (partly) as drug metabolites, which can be pharmacologically active or inactive. Deriving f_{ex} can, however, be challenging due to the complexity of human metabolism and the fact that available data on drug metabolism and excretion are not always reported in a consistent or complete manner. **Methods:** With a team of specialists from various domains, we established a systematic pharmacokinetic data retrieval and assessment framework. It considers Summary of Product Characteristics (SmPC) documents as a principal source of up-to-date drug information due to their regulatory status. SmPCs subsequently aid in the identification of detailed data on drug metabolism and excretion, mostly in marketing authorization documents and scientific study publications. Relevant information can then be extracted following the structured assessment process we developed and tested using various therapeutic drugs of environmental interest. **Results:** Our workflow was successfully developed and tested, demonstrating its use in extracting and interpreting pharmacokinetic information relevant for deriving f_{ex} . During testing, however, several considerations emerged, including: whether to use f_{ex} in the first place, incomplete recovery and healthy (male) volunteer bias in mass balance studies, and an apparent underreporting of phase II metabolites in older mass balance studies. A preliminary comparison between f_{ex} values derived using our approach and those used in recent drug emission studies furthermore revealed substantial differences in the values obtained, despite all approaches relying on SmPCs as key source documents. **Conclusion:** We developed a systematic workflow to extract human pharmacokinetic data from regulatory documents and mass balance study publications. This workflow allows for transparent derivation of excretion fractions for use in drug emission models.

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3.21.T-03 Degradation products of siRNAs and ASOs following incubation in sewage sludge

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Small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) are two types of a new pharmaceutical modality. These oligonucleotide therapeutics feature chemical modifications such as 2'-O-methyl and 2'-fluoro substitutions, Locked Nucleic Acid (LNA) bases, phosphorothioate linkages, and 5-methyl cytosine, that improve stability and reduce nuclease susceptibility, with potential implications for environmental persistence following excretion into wastewater systems.

We first conducted a miniaturized study in microtubes to assess degradation under realistic wastewater conditions. A GalNAc-conjugated siRNA duplex targeting HPRT and its antisense strand were incubated in sewage sludge (2500 mg TSS/L) in non-aerated microtubes for 7 days. Fluorescent probes confirmed active endonuclease and exonuclease activity. High-resolution mass spectrometry showed the siRNA duplex disappeared by 93.6% within 24 hours and was undetectable by Day 7. The antisense strand exhibited similar disappearance (98.2% on Day 1) and was also undetectable by Day 7. Mass-MetaSite analysis identified 25 fragments (5–23 nucleotides), confirming enzymatic cleavage.

To validate these findings and assess biodegradability, we initiated 28-day OECD 301B and 314B studies. Test substances included the HPRT siRNA duplex, its antisense strand, and a MALAT1-targeting ASO, all with chemical modifications. Sludge concentrations were 30 mg TSS/L for 301B and 2500 mg TSS/L for 314B. Samples were collected at multiple time points throughout the 28-day period for HRMS analysis (pending final results).

Nuclease activity was consistently higher in 314B sludge. In OECD 314B, endonuclease activity was $6.58 \times 10^{??} \mu\text{mol}/\text{min}$ —comparable to the preliminary study ($1.34 \times 10^{??} \mu\text{mol}/\text{min}$)—demonstrating little evidence of seasonal variability. 301B sludge showed much lower activity ($2.94 \times 10^{??} \mu\text{mol}/\text{min}$), ~22-fold lower than 314B, likely due to the diluted sludge.

Chemically modified oligonucleotides were not readily biodegradable by OECD 301B criteria, showing negligible CO₂ evolution despite ready biodegradability of unmodified RNA controls. However, realistic wastewater conditions (2500 mg TSS/L) and analysis by HRMS demonstrated that chemically modified therapeutic oligonucleotides are highly susceptible to rapid, repeated primary degradation by both endo and exonucleases.

3.21.T-04 Projected Ecotoxicological Risks from Pharmaceuticals in German Rivers by 2050

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The European Union (EU)'s zero-pollution ambition targets a toxic-free environment by 2050, but an aging population and a warming climate increase disease prevalence and pharmaceutical use, leaving future pollution levels and the route to this goal uncertain. Using a node-link model informed by Representative Concentration Pathways (RCP) and Shared Socioeconomic Pathway (SSP) scenarios, we projected concentrations of five pharmaceuticals and their ecological risks to three aquatic species in 1-km river stretches across Saxony, Germany, by the 2050s. Signal-to-noise ratios ranged from 4.49 ± 1.30 (carbamazepine) to 7.68 ± 2.22 (ciprofloxacin), showing an agreement across 15 climate models. Under SSP5–8.5, pharmaceutical concentrations varied from ~98% (sulfamethoxazole) to 192% (gabapentin) without policy intervention in pharmaceutical prescribing, and from ~60% (carbamazepine) to ~23% (metformin) with stringent policy intervention, relative to the 2010s baseline. Rivers under at-risk toxicity from pharmaceutical mixtures increased by 7.7% (algae) to 12.4% (daphnia) without intervention, but decreased by 6.5% (daphnia) to 6.8% (fish) with intervention. To reach no-harm targets for the ecosystem from pharmaceutical mixtures at the moderate-risk, 21% (with intervention) to 33% (without intervention) of wastewater treatment plants require full upgrades for all pharmaceuticals. This study consolidates knowledge on the gaps to EU's zero-pollution goals amid socioeconomic and hydroclimatic changes.

3.21.T-05 A Framework to Identify High-Priority Legacy Active Pharmaceutical Ingredients for Environmental Risk Assessment

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Active Pharmaceutical Ingredients (APIs) from human medicinal products are detected in surface waters globally, but the full extent of their impact remains insufficiently understood. Conducting environmental risk assessments (ERAs) including experimental studies for all pharmaceuticals is considered unfeasible, particularly for legacy APIs approved before the ERA became a mandatory requirement in the European Union. This challenge can be addressed by prioritising legacy APIs for ERA, as highlighted by the European Commission's recent proposal for a revised EU pharmaceutical legislation, stating the need to establish scientific criteria to prioritise legacy APIs, using a risk-based approach.

In line with this, we present a risk-based prioritisation framework to identify high-priority legacy APIs for further testing and ERA, which we applied to a dataset of 1,687 APIs. Predicted environmental concentrations in surface waters (PEC_{sw}) were estimated using European consumption data from 2019 to 2023. Effect concentrations were predicted using multiple approaches, including Quantitative Structure-Activity Relationship (QSAR) models, and Theoretical therapeutic Water Concentrations (TWCs). To validate these predictions, QSAR and TWC values were compared against empirical ecotoxicity data for a subset of data-rich APIs. Results showed that QSAR models, which generally show acceptable statistical performance, tend to underestimate the effects of highly toxic pharmaceuticals. In contrast, TWC values were more conservative but exhibited greater variability in performance. Exposure and effect concentrations were combined into preliminary risk quotients (pRQs), which were ranked and subsequently benchmarked against a reference ranking for validation. In the final step, multiple pRQs were combined into a single risk-based ranking. This list, along with a summary of ERA data availability for the included APIs, can serve as a starting point for stakeholders, such as industry and regulators, to screen legacy APIs for data gaps, guiding decisions on which substances require a further ERA, possibly involving testing, and what types of studies are needed.

3.21.P Pharmaceuticals in the Environment – Innovations in Risk Assessment, Regulation, and the Science Globally

3.21.P-Tu347 A Step Towards Understanding the Global Environmental Distribution of Aquatic Exposure from Consumer and Institutional Pharmaceutical Usage

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Improved environmental risk assessment and regulation for pharmaceuticals requires that we are better able to predict and mitigate movement into surface water. It is important to ensure that water stewardship, problem identification, prioritization and action are science based. The pharmaceutical industry faces scrutiny regarding the environmental impact of APIs that can enter water systems from consumer use at home, institutional uses in hospitals, manufacturing sites, and animal health uses. Companies must be aware of the eventual emissions and potential impacts of ingredients. Mapping of detailed water network emissions empowers companies to predict the distribution of their ingredients in various aquatic ecosystems. This prediction helps in developing robust environmental risk assessments and management plans ensuring readiness for increased regulations and mandatory disclosure. Accurate and robust information on water vulnerability supports regulatory agencies in their remit for the protection of human and environmental health. We developed a globally consistent, spatially explicit freshwater exposure model for down-the-drain substances, with an initial focus on pharmaceuticals. The EmiE model (Evaluating Micropollutants in the Environment) is built upon established datasets at global and national levels, spatially linking sources of pharmaceutical uses, emissions and receiving water systems. Beyond consumer uses, we have added the ability to model localized high-intensity emissions from hospitals and other institutions. The model has been designed for extensibility, with an eye towards including manufacturing emissions as well as diffuse source emissions (e.g., from veterinary uses). This holistic view of emissions enhances our understanding of potential environmental impact, allowing for more proactive management and more localized and effective mitigations. While the model operates at the finest level of hydrologic resolution (the river segment), embedded tools supply seamless aggregation to multiple basin levels using a choice of

metrics, allowing for direct linking of model results to other existing environmental protection efforts. Whether mitigating pollution, ensuring compliance, optimizing resource management, or protecting ecosystems, trusted and transparent maps and predictive tools are indispensable for businesses committed to operating responsibly in an evolving global landscape.

3.21.P-Tu348 Case study on the significance of persistent and mobile pharmaceutical active substances in drinking water from bank filtrate – against the backdrop of the Urban Waste Water Treatment Directive

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Innovations in European water legislation may have a long-term positive impact on the entire water cycle, provided they are properly implemented in the Member States. With the latest versions of the EU Urban Waste Water Treatment Directive (UWWTD) and the EU Drinking Water Directive (DWD), which now also emphasises the importance of drinking water catchment areas, ‘from source to tap’ seems to be more relevant than ever in terms of water quality. UWWTD states that human pharmaceutical and cosmetic products are particularly problematic in terms of the formation of micropollutants in water bodies.

In our case study, we thus investigated the actual influence of upstream waste water treatment plants (WWTP) on the pharmaceutical spectrum throughout the entire water cycle at a bank filtrate-fed drinking water production site: from the WWTPs effluents through the receiving river and the bank filtrate to the finished drinking water. Due to their different dimensions, the respective WWTP are not yet equally affected by the requirements of the UWWTD, but already differ significantly in the treatment technology applied. Water samples taken from wastewater effluents, surface water, groundwater, abstraction wells and treated drinking water were analysed using high-performance liquid chromatography coupled with tandem mass spectrometry. A total of around 30 pharmaceutical active substances were analysed, with the focus later being placed on examining the occurrence and behaviour of five representative compounds, that were particularly prominent in drinking water samples, including the metabolite oxipurinol. Individual pharmaceutical residues were detectable throughout the entire water cycle, right up to the finished drinking water.

The results provided evidence of the contamination pathway from the sewage treatment plant to the drinking water for the case study area. Persistence and mobility proved to be particularly relevant properties for this contamination pathway, with the persistent and mobile oxipurinol and the UWWTD indicator substance carbamazepine epitomising this problem.

Although bank filtration offers significant natural protection against certain trace substances, pharmaceutical residues, waste water discharges remain the main source of river contamination and, subsequently, the groundwater system. These results are highly relevant to the implementation of the revised UWWTD, which prioritises an additional fourth treatment stage to be implemented.

3.21.P-Tu349 Environmental Occurrence and Antimicrobial Resistance Potential of Carbapenems – Last-Resort Antibiotics

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The misuse of antibiotics in human and veterinary medicine is a major driver of antimicrobial resistance (AMR). Although antibiotics are widely detected in aquatic environments, monitoring of the last-resort β -lactam antibiotics – carbapenems – remains extremely limited. Their structural similarity to penicillins and partial excretion in humans suggest that carbapenems enter wastewater and surface waters, potentially exerting selective pressure that promotes environmental AMR. However, empirical evidence has been scarce.

In this study, we developed and validated a sensitive liquid chromatography–tandem mass spectrometry (LC-MS/MS) method to quantify six carbapenems (biapenem, doripenem, ertapenem, faropenem, imipenem and meropenem) in environmental waters. Applying this method to Danish hospital effluents and surface waters.

Because carbapenems are chemically unstable and may form biologically active transformation products (TPs), we further examined their fate under environmentally relevant conditions. TPs of ertapenem, faropenem, imipenem and meropenem were observed, and their potential contribution to AMR is under evaluation.

By analyzing both parent compounds and transformation products, this work provides new evidence of the overlooked environmental persistence and transformation of carbapenems. These findings highlight their potential role in sustaining selective pressure for AMR in aquatic environments.

3.21.P-Tu350 State of Pharmaceutical Pollution in Italian Waters and Estimation of the Load Discharged from Rivers into Coastal Waters

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In recent decades, the combination of “pharmaceuticals” and “personal care products” (PPCPs) has been recognised as a contaminant of “emerging concern” due to their persistent presence in the environment. As part of a study on the risk assessment of PPCPs in Italian waters, we conducted a sampling campaign to detect the presence of pharmaceutical compounds, including active ingredients and excipients, in Italian waters. We collected more than 150 samples of surface water (rivers, lakes and reservoirs), groundwater and spring water at approximately 120 sites. Approximately 30 rivers flowing into the Tyrrhenian Sea, the Adriatic Sea and the Ionian Sea in 12 Italian regions from north to south were sampled at the end of the basin, near the river mouth, in order to estimate the overall drug load in rivers flowing into Italian coastal waters. We developed a screening method based on solid-phase extraction and high-resolution HPLC (Orbitrap) mass spectrometry to assess the presence of pharmaceuticals, followed by the quantitative determination of over 80 compounds. The picture that emerged from the study showed that the problem of pharmaceuticals is widespread throughout the country, with some compounds (e.g. metformin, caffeine, sucralose and carbamazepine) often determined at microgram-to-tens of microgram levels. The combination of screening and quantitative determination methods made it possible to assess the state of pharmaceutical pollution in Italian waters and to highlight the different sources, including urban and hospital wastewaters. In addition, the quantitative data were compared with the available PNECs in order to assess the specific risk of the monitored sites and using the risk quotient approach.

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3.21.P-Tu351 A Pragmatic Approach to Determine Rapid Degradability of Cosmetic and Pharmaceutical Substances Under the European Union's Urban Wastewater Treatment Directive

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The European Union's new Urban Wastewater Treatment Directive (UWWTD) will require extended producer responsibility (EPR) fees for companies in the pharmaceutical and cosmetics sectors when their products contain substances defined as “micropollutants.” However, the Directive's definition of micropollutants excludes “rapidly degradable” substances. For the definition of “rapidly degradable” substances, the Directive refers to the Classification, Labelling and Packaging (CLP) Regulation. However, the application of the CLP definition for the purposes of implementing the UWWTD lacks clarity. Therefore, we will assess the CLP definition, the underlying test methods, and associated data to determine “rapidly degradable” substances for the UWWTD and provide a pragmatic approach to avoid unnecessary testing burden while ensuring environmental protection.

We propose a tiered approach to exclude substances from the UWWTD's scope due to biodegradability using the results of first, screening tests and second, simulation tests. Abundant Ready Biodegradability study results exist for many substances, which can provide valuable insights. Instituting completely new testing methods solely for the UWWTD, as some stakeholders have suggested, would require considerable resources to standardize these methods and conduct tests on numerous substances that already have biodegradability data available. Ready (and Inherent) Biodegradability tests are screening tests that are designed to be stringent and difficult to pass and are intended as indicators of rapid and ultimate degradation in most environments, including biological wastewater treatment plants (WWTPs). Therefore, some substances that fail these screening tests may actually be rapidly degradable under more favorable conditions of typical WWTPs. Consequently, assigning EPR fees simply based on the failure to meet the ready biodegradability criteria would be inappropriate, violating the polluter pays principle, i.e., the polluter should bear the expenses of preventing and controlling pollution. We advocate for the possibility of stakeholders to perform more realistic higher-tier studies that can override lower-tier screening studies. These optional higher-tier studies should be based on either (1) well-established simulation tests (e.g., OECD Methods 303A and 314b), or (2) a combination of in silico models (e.g., SimpleTreat) and environmental monitoring at the outfall of properly operating tertiary WWTPs.

3.21.P-Tu352 Photocatalytic removal of aqueous PPCPs with In(OH)3-InVO4 modified carbon nitride
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In recent years, environmental problems caused by medicines and personal care products (PPCPs) have attracted much attention. Heterogeneous photocatalytic technology has become a research hotspot due to its characteristics of not requiring the addition of soluble oxidants and efficient degradation of PPCPs. In this study, the In(OH)3-InVO4/g-C3N4 catalyst was synthesized by the hydrothermal method. It presented a mesoporous structure. Compared with g-C3N4, the functional groups did not change significantly, but the specific surface area increased significantly. The light absorption capacity, light response performance and separation efficiency of photogenerated carriers were all significantly enhanced. The removal efficiency of OTC after 60 minutes of light exposure reached approximately 91%. The coexistence of HCO3⁻ significantly enhances the removal efficiency of OTC by promoting the generation of carbonate radicals. Meanwhile, a higher pH can promote the generation of superoxide free radicals ($\cdot\text{O}_2^-$), thereby enhancing the treatment efficiency. The active species capture experiment confirmed that holes (h^+) and $\cdot\text{O}_2^-$ were the dominant active species in the system. Based on the characterization analysis and inference, the construction of the S-type heterojunction promotes the effective separation of electrons (e^-) of the g-C3N4 conduction band and holes of the In(OH)3-InVO4 valence band. h^+ directly participates in the oxidation of pollutants, and the edge potential of the g-C3N4 conduction band is greater than the potential of $\text{O}_2/\cdot\text{O}_2^-$ (-0.33 eV). Further drive e^- to reduce O_2 to $\cdot\text{O}_2^-$, synergistically enhancing the degradation of pollutants.

3.21.P-Tu353 Distribution and Ecological Risk Evaluation of Pharmaceuticals in Catalanian Rivers (Spain)

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Pharmaceutical residues are increasingly detected in surface waters due to growing consumption and the limited removal capacity of conventional wastewater treatment plants. Their persistence can affect aquatic organisms and may compromise ecosystem functioning. Catalonia, a region with high population density and diverse river basins, has not previously been studied at this extent. Most research has focused mainly on the Besòs and Llobregat because of their proximity to the Barcelona metropolitan area. Understanding the extent of contamination and its ecological implications is essential for improving water quality management.

This study aims to characterise the occurrence of pharmaceutical residues in Catalanian rivers and evaluate their ecological risks. To achieve this, a broad monitoring campaign was conducted across 14 major rivers, encompassing 52 sampling locations from sources to mouths. In total, 72 pharmaceuticals and five metabolites were quantified using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to detect trace concentrations. Ecological risks were assessed through risk quotients, calculated for algae, invertebrates and fish, providing an integrated view of potential impacts across trophic levels.

Pharmaceuticals were detected in almost all rivers, although eight compounds were not found in any sample. The highest concentrations occurred in basins influenced by urban, industrial or wastewater discharges, particularly the Besòs and Tordera. Several pharmaceuticals exceeded ecological risk thresholds, especially for algae, which emerged as the most sensitive group. Pentoxifylline and fluoxetine showed the greatest risk potential, while levofloxacin was the main concern for invertebrates. Areas of elevated risk were mainly located at river mouths or downstream of densely populated zones.

The study concludes that pharmaceutical contamination is widespread and ecologically relevant. These findings provide evidence to support improved water management in Catalonia. The results identify priority compounds and highlight specific locations where mitigation or enhanced monitoring should be implemented. By providing the first region-wide overview of pharmaceutical pollution, this work advances understanding of the issue and offers practical guidance for authorities and water managers to develop effective measures to protect river ecosystems.

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3.21.P-Tu354 Can Bioaccumulation Screening Based on Log KOW Associated with the Neutral-Only Forms for Ionizable Substances Drive Unnecessary Fish Testing?

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The European Medicines Agency (EMA) Environmental Risk Assessment (ERA) Guideline requires pH-corrected octanol-water distribution coefficients (Dow) for ionizable active pharmaceutical ingredients (APIs) to be compared against octanol-water partition coefficient (Kow) screening criteria for bioaccumulation assessments. Given that the Dow for ionizable APIs are determined at three pH levels sufficiently encompassing the environmentally relevant pH range, concerns were raised with the Agency that further pH-correction is not justified and leads to unnecessarily conservative screening and subsequent need for redundant animal testing. However, the regulatory requirement remains in effect. Furthermore, EMA provides a specific approach for pH-correction only for monoprotic APIs (i.e., with a single dissociation constant) and not for APIs with multiple dissociation constants.

This study will evaluate the validity of the rationale requiring pH-correction of the log Dow values determined at three pH levels encompassing the environmentally relevant range (pH 5 to 9) for ionizable APIs with both single and multiple dissociation constants. Publicly available log Dow and bioaccumulation data will be evaluated, as well as recent literature on conceptually similar pH-correction applied to ecotoxicity of ionizable substances. Additionally, this study will show how the narrower optimal pH range for definitive in vivo test organisms for bioaccumulation testing (e.g., OECD Methods 305 and 321) may limit the practical usefulness of the currently required screening over a wider pH range.

The results of our evaluations will inform a more appropriate bioaccumulation screening approach based on log Dow values for ionizable substances.

3.21.P-Tu355 Assessing the Environmental Fate of a New Promising Antimicrobial Peptide

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Antibiotics are widely used in human and veterinary medicine, and have contributed significantly to the increase in human lifespan over the last century. However, their overuse has led to the development of antimicrobial resistance (AMR), a major global health threat. Once excreted, antibiotics and their transformation products are emitted into the environment, where they can impact human and environmental health by promoting AMR or by exerting other hazardous impacts. Characterization of their environmental fate allows to evaluate such effects and is therefore required. As with others organic contaminants, the fate of antibiotics in ecosystems is driven by processes such as hydrolysis, photolysis, biodegradation or adsorption. In order to tackle AMR, it is necessary to develop new antibiotics less susceptible to create resistance. A promising antimicrobial peptide, Pep16, was synthesized for this purpose. Its environmental fate and transformation products were characterized using batch experimentations simulating environmental conditions, to ensure that the possible future large-scale use of this compound will not present risks to human and environmental health. Among the investigated processes, hydrolysis was not observed in ultrapure water, but was observed in river water. Water matrix constituents are responsible for this difference, especially Cu²⁺ which was shown to catalyze Pep16 degradation. Direct and indirect photolysis of Pep16 were assessed, and the effect of light attenuation depending on immersion depth in river water will be further characterized for better environment representativeness. Adsorption was shown to be important on the tested sediments. Kinetics and isotherms variations were highly depending on their physicochemical properties. Biodegradation of Pep16 by microbial communities from river water and raw wastewater was relatively fast and was found to be fluctuating with season and experimental conditions variations. The applied methodologies are contributing to the evaluation of Pep16 environmental fate. The knowledge acquired on the processes related to the fate of Pep16 is sufficient to predict its persistence, which will be relatively limited and controlled by the physicochemical properties of the environment, and its mobility, which will be limited due to strong adsorption. This work is therefore essential for evaluating the potential impacts of Pep16 on human and environmental health if it were to be marketed.

3.21.P-Tu356 Challenges of pH adjustment of toxicity tests for ionisable pharmaceuticals

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New guidance for the environmental risk assessment (ERA) of pharmaceuticals was published in February 2024. As many pharmaceuticals are ionisable across an environmentally realistic pH range, and because

bioavailability and therefore toxicity may vary with pH, the new guideline includes a requirement for toxicity testing to be conducted “at a stable pH consistent with the most bioavailable form of the test chemical”. This will usually be the neutral or non-dissociated form. Standardised ecotoxicity tests have been optimised to be conducted under water quality conditions which maximise survival and performance of the test organisms within the assays. Some of the challenges that have been encountered when attempting to adjust the pH of media in a fish early life stage (ELS) test and a sediment water toxicity test with *Chironomus riparius* will be discussed. For the example test chemical the aim was to maintain the pH in the test system between pH 6 and 7. In the ELS the pH of the dilution water was adjusted to 6 using 37% hydrochloric acid to reduce the pH of the stock dosing solutions. During the test the pH of test vessels was maintained between 6.65 to 6.92. The test validity criteria were met but post hatch survival was 76% (guideline criteria is 75%). This is lower than is typical for the laboratory (historical control value is 79-97% (mean = 90%) for the last 11 studies). For the chironomid tests adjusting the pH of the test media alone was insufficient to maintain the test pH. The sediment was therefore also adjusted to pH 6 (sediment pH during the test was 6.43-6.48). However, the test validity criteria were not met for this test indicating that the test organisms did not tolerate the lower pH. In addition, the sediment carbon concentrations did not meet the levels recommended for the test because sediment pH is adjusted using calcium carbonate (i.e. an additional carbon source). There is a fine balance between conducting ecotoxicity tests at a pH consistent with the most bioavailable form of the test chemical and fulfilling test validity criteria. In addition, it can also be unclear if any increased sensitivity observed in the tests is due to maximised test substance bioavailability or because the test animals are under additional stress. pH adjustment of toxicity tests is expected to lead to more repeated tests which has ethical implications for vertebrate testing.

3.21.P-Tu357 pH-Dependent Toxicity of the Ionisable Pharmaceutical Ibuprofen in Aquatic Environments

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Concern around the toxicity of ionisable molecules in relation to the pH of environmental lotic waters compared to testing media has been raised under regulatory frameworks (e.g. EMA ERA guidelines and Water Framework Directive EQS derivations), and in the scientific literature. For example, studies such as Chang et al. 2021 have raised this concern in relation to several active pharmaceutical ingredients including ibuprofen, although these findings were based on fish gill cell culture model and little data exists in intact organisms in environmentally relevant conditions. Given that the EMA ERA guidelines and EQS conclusions have significant regulatory impacts, further studies are useful to fully contextualise risk.

In EU lotic waters, pH typically ranges from 6 to 8, with more than 93% of sites above pH 7. Conducting aquatic toxicity assessments under altered pH conditions in laboratory settings is often impractical and experimentally complex. Such modifications can result in non-compliant tests because permissible pH ranges for test media are constrained by OECD guidelines and may vary according to species-specific or media-specific requirements. As a result, studies on aquatic organisms completed as part of regulatory processes consider the toxicity at species specific pH, usually around pH 7.

Since ibuprofen is a weak acid with a pKa of 5.15, its toxicity could theoretically increase with decreasing water pH. It should be noted that in any environmentally relevant EU lotic water ibuprofen will never reach 50% ionisation nor be fully ionised. To investigate the effect of modulating pH on ibuprofen toxicity to intact organisms, several studies were conducted. Fish, the most sensitive aquatic organism to ibuprofen, were tested in an OECD 236 fish embryo toxicity test with media adjusted to environmentally relevant pH levels. Two OECD 211 daphnia reproduction studies were also conducted with test media at varying pH levels and provide supplementary information.

Across all studies, ibuprofen toxicity was unaffected by variations in test media pH, indicating no biologically or statistically significant increase in toxicity within environmentally relevant pH ranges. Since these studies were all OECD guideline compliant and to GLP and C1, R1 according to CRED evaluation, the results also provide robust regulatorily relevant endpoints for consideration under ERA and EQS evaluations of ibuprofen in the future.

3.21.P-Tu358 Innovating Aquatic Toxicology: Lessons Learned from a Novel Full Life Cycle Fish Study

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Robust chronic ecotoxicological data are critical for pharmaceutical registration within the EU, as outlined by the European Medicines Agency (2024), and plays a pivotal role in policy development and regulatory decision-making. The EQS dossier SCHEER (2023) and Faber et al. (2023) highlights that although several freshwater toxicity studies on ibuprofen exist, many fail to meet CRED scoring criteria, resulting in notable gaps and uncertainties when assessing and drawing conclusions on the chronic aquatic toxicity profile of Ibuprofen. Oldenkamp et al. (2022) similarly stressed the importance of high-quality, accessible ecotoxicity data on pharmaceuticals to support scientific research, regulatory frameworks, and public awareness.

The authors used a collaborative approach that included consultation with leading academic experts and regulatory authorities to develop a full life cycle test suitable for investigating the toxicological effects of Ibuprofen to fish (fathead minnows, *Pimephales promelas*) with the intention to continuously expose F0 embryos (<24-hours old) through all growth and reproduction stages to the production and growth of F1 juveniles.

The full life-cycle study on fathead minnows was conducted in compliance with Good Laboratory Practice (GLP) and, where applicable, OECD guidelines to ensure methodological rigor. Given the novelty of this approach, the study provided a unique opportunity to observe fathead minnows over an extended duration in the laboratory. The extensive dataset generated encompassed biological and behavioural parameters as well as operational aspects of the test system. These observations offer valuable insights for the design and execution of future long-term studies as well as interpretation of the results observed. Several challenges encountered during the study prompted consideration of procedural refinements, while others emerged from the interpretation of behavioural data, informing potential considerations of aspects of the study guidelines. The poster discusses these challenges and proposes solutions and recommendations for subsequent research.

3.21.P-Tu359 Ecotoxicological Impacts of the Painkiller Indomethacin on *Procambarus clarkii*: Tissue-specific Cytotoxic and Oxidative Responses

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Non-steroidal anti-inflammatory drugs (NSAIDs) are increasingly acknowledged as emerging contaminants in freshwater environments, but their impacts on aquatic invertebrates are still not well understood. This study provides the first combined assessment of cellular and biochemical responses in the red swamp crayfish (*Procambarus clarkii*) following long-term exposure to indomethacin, a commonly used NSAID. Individuals were exposed for 28 days to concentrations of 2.5, 5, and 10 mg/L, and a suite of cytotoxicity and oxidative stress biomarkers was examined.

Cell viability in hemolymph and hepatopancreas was evaluated using Trypan Blue and Neutral Red assays, while oxidative stress indicators were measured in hepatopancreas, gills, and muscle: superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST), and malondialdehyde (MDA). Results showed that indomethacin elicited notable cytotoxic and oxidative responses that varied across tissues. Importantly, hepatopancreas cell viability declined significantly at lower concentrations, with partial recovery at the highest dose, suggesting a potential hormetic effect. Oxidative stress markers showed concentration-dependent shifts: SOD and MDA were significantly modified in the hepatopancreas, GST activity changed in the gills, and GPx activity increased in muscle at the highest concentrations. Hemocyte viability remained stable, indicating a degree of resilience in circulating cells. The Integrative Biomarker Response-Threshold (IBR-T) index revealed both hormetic and traditional dose-response patterns depending on the tissue. From an ecological standpoint, these findings indicate that *P. clarkii* may withstand relatively high levels of indomethacin without undergoing systemic oxidative failure, potentially contributing to its persistence and competitive success in contaminated environments. However, the sensitivity of the hepatopancreas points to possible sublethal metabolic disturbances that could influence long-term fitness and population dynamics. Overall, this research offers new insights into the ecotoxicological effects of indomethacin in a key invasive species and underscores the value of tissue-specific biomarker approaches in environmental risk assessment.

3.21.P-Tu360 Consumption of Microalgae Exposed to Diclofenac Affects the Survival and Reproduction in the Cladoceran *Ceriodaphnia dubia*

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Diclofenac (DCF) is a nonsteroidal anti-inflammatory drug (NSAID) with antipyretic and analgesic effects; worldwide, its approximate annual consumption is 2,400 tons. DCF is the most widely used NSAID in humans and animals for treating muscle pain and inflammatory conditions. It is marketed as oral tablets or topical gel and is sold over the counter under various brand names. Urban and hospital wastewater are the primary sources of DCF release. Its presence in water bodies is not regulated, and concentrations ranging from 0.02 ng/L¹ to 216 µg/L¹ have been detected, which can negatively impact aquatic biota and its trophic structure. Therefore, the objective of this study was to evaluate the effect of food exposed to diclofenac and potentially contaminated by this drug, on the reproductive parameters of *Ceriodaphnia dubia*. The microalga *Pseudokirchneriella subcapitata* was exposed to different concentrations of DCF (12.5, 25, and 50 mg L⁻¹) in Bold Basal Medium for 7 days, and subsequently used as food for *C. dubia*. The median lethal concentration (LC50) of DCF was determined in the cladoceran at 48 h. Subsequently, a 15-day chronic bioassay was performed, supplying *C. dubia* 1 x 10⁷ cells mL⁻¹ of *P. subcapitata* without diclofenac (control) and 1 x 10⁷ cells mL⁻¹ of *P. subcapitata* as the intoxicated food obtained from each DCF concentration. Survival and reproductive responses of *C. dubia* (total progeny, age at first reproduction, and number of clutches) were evaluated daily. The LC50 of diclofenac for *C. dubia* was 58.56 mg L⁻¹. Cladoceran survival decreased by 10% with the contaminated feed. Reproduction (total progeny) of *C. dubia* decreased significantly compared to the control in treatments fed with microalgae exposed to DCF, and the age at first reproduction was delayed by up to 1 day at the highest concentration (50 mg L⁻¹). These findings highlight the importance of considering the effects of emerging contaminants, such as DCF, on the trophic structure of the aquatic environment. The planktonic cladocerans, as non-selective filter feeders, are directly exposed to toxic substances in the water and, indirectly, through the ingestion of microalgae that are also exposed to and possibly affected by these chemical pollutants, which can act as toxic carriers and become an additional environmental stressor.

3.21.P-Tu361 Temperature Affects the Toxicity of Amoxicillin in *Microcystis aeruginosa* and Modifies the Toxicogenicity of This Cyanobacterium

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The quality and availability of freshwater are currently severely affected by population growth, urbanization, and intensified agricultural practices, which lead to excessive discharge of mineral nutrients into aquatic environments. This, coupled with climate change, contributes to increased intensity and frequency of harmful cyanobacterial blooms, mainly dominated by *Microcystis aeruginosa*, with negative repercussions for aquatic life and humans due to the synthesis and release of cyanotoxins. Furthermore, contaminants of emerging concern, such as pharmaceuticals and especially antibiotics, influence cyanobacterial growth. Globally, in 2024, the production of amoxicillin trihydrate was estimated at approximately 69,000 tons for use in human and veterinary medicine. This antibiotic has been detected in aquatic ecosystems at concentrations ranging from ng to µg L⁻¹. Therefore, the objective of this study was to evaluate the effect of amoxicillin and two temperatures (25 and 30 °C) on the growth and concentrations of photosynthetic pigments, phycobiliproteins, and cyanotoxins of *M. aeruginosa*. The Median Inhibitory Concentration (IC50) was determined according to OECD Protocol 201. Subinhibitory bioassays were performed with 50,000 cells mL⁻¹ in BG11 medium at two temperatures (25 °C and 30 °C), exposing the cells to amoxicillin concentrations of 102, 188, and 426 ng L⁻¹, with constant aeration and continuous illumination at 30 µmol photons m⁻² s⁻¹ for 96 h. The IC50 of amoxicillin in *M. aeruginosa* was 544.53 ng L⁻¹. In bioassays evaluating the effect of temperature on amoxicillin toxicity, cell growth was comparatively more inhibited at 30 °C, but pigment synthesis, particularly carotenoids, phycocyanin, and allophycocyanin, was significantly stimulated. Regarding cyanotoxin synthesis, the highest concentration of microcystins was recorded in *M. aeruginosa* at 30 °C when exposed to the highest amoxicillin concentration (426 ng/L). Our results demonstrate that amoxicillin alters the development of *M. aeruginosa* and that higher temperatures intensify these effects, favoring the synthesis and accumulation of microcystins, which pose a potential risk to biota and humans. Therefore, the importance of considering the combined effects of pharmaceutical contaminants and global warming in freshwater toxicity studies is highlighted, as these factors can influence other processes, such as eutrophication, which, in turn, can amplify the impacts of emerging contaminants.

3.21.P-Tu362 Cytostatic pharmaceuticals: from regulatory dossiers to real ecotoxicity data.

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Pharmaceuticals are widespread in aquatic ecosystems. This unintended load is known to potentially affect aquatic organisms. To control the entry of pharmaceuticals in the market in the European Economic Area (EEA) authorization is a mandatory prerequisite, including an Environmental Risk Assessment (ERA) of the active pharmaceutical ingredients (APIs).

Antineoplastic agents have been among the most frequently authorized APIs in the EEA over the past decade, these are mainly use in cancer treatment. However, the ecotoxicological information included in their authorization dossiers remains limited, resulting in a lack of regulatory-compliant ecotoxicological data for these APIs.

This work focuses on cyclophosphamide and ifosfamide which are classified as cytostatic antineoplastics APIs, which generally intervene in cell division, and their main role is to inhibit or completely block the replication of DNA. The toxicity of these two cytostatic substances will be assessed by using single-species bioassays guidelines for different model organisms, in order to represent the effects in the ecosystem at different trophic levels.

In aquatic organisms the toxicity on primary producers growth inhibition has been assessed in *Pseudokirchneriella subcapitata* following OECD 201 guidelines. Also, acute and chronic toxicity on secondary producers has been assessed using *Daphnia magna*. For the acute toxicity the target endpoint was survival following OECD 202 guideline. As for the chronic toxicity were survival, reproduction and growth by following OECD 211 guideline. Additionally, sediment effect assessment has been assessed in chironomids emergence following OECD 218 and ostracods survival and growth according to ISO 14371.

The aim of this study is to understand the potential toxic effects of cytostatic substances in the aquatic ecosystems. Also, to provide good quality and regulatory compliant ecotoxicity data for model organisms.

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3.21.P-Tu363 Ecotoxicological Effects of Natural Antimicrobial Terpineol on Soil Bioindicators and Microbial Communities

Laura Botello-Morte, Antonio Valenzuela, Elisa Langa, Pedro Rodríguez-López, Diego Ballester and María Rosa Pino Otín, San Jorge University, Spain

Terpineol (TN) is a monoterpene naturally present in essential oils (EOs) from aromatic plants or obtained through biotransformation. Its aroma and solvent properties make it a popular in both the chemical and food industry as an ingredient and additive, respectively. Furthermore, it presents some therapeutic properties such as antimicrobial activity against pathogenic or multidrug resistant (MDR) bacteria.

As a result, global demand is increasing and despite being generally recognized as safe (GRAS) compound for humans, information regarding the potential environmental impact is still scarce. Since it is assumed to be fully biodegradable in water, it would be interesting to evaluate the impact of the use of terpineol over soil. In this study, the ecotoxicity of terpineol using key soil bioindicator organisms (*Allium cepa* and *Eisenia fetida*) and soil microbial communities was assessed.

The *A. cepa* assay yielded a 72 h EC₅₀ of 50.555 (40.807-63.574) µg/mL, while the 14-day *E. fetida* assay resulted in a LC₅₀ of 55.791 (47.769-62.141) mg/kg, suggesting a moderate toxicity of TN towards individual soil bioindicators, that increased in a dose-dependent manner. Additionally, the effects of TN on soil microbial communities were evaluated to provide a more ecologically relevant perspective. Terpineol at 10 and 100 µg/mL significantly reduced microbial growth after 72 h hours of incubation. Additionally, TN caused a dose-dependent reduction in microbial metabolic activity, presenting statistical significance when amines or amides were used as carbon source at 10 µg/mL ($p < 0.05$) and 100 µg/mL ($p < 0.01$) of TN added.

These results highlight that TN, despite being considered biodegradable and low-risk for humans, may pose ecological risks when released into soil environments. Regulatory consideration and further research are needed to better understand its long-term environmental effects and to support safe use guidelines.

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3.21.P-Tu364 Enhanced Ecotoxicity of Synergistic Antibiotic and Natural Compound Combinations: An Aquatic Outlier

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The widespread emergence of antibiotic-resistant bacteria has driven the search for alternatives, including the synergistic use of commercial antibiotics (ATBs) and natural compounds (NCs). Although promising against pathogens, their combined environmental fate and ecotoxicity on non-target aquatic organisms remain largely unknown. This research addresses the critical ecotoxicological risk posed by seemingly benign NC-ATB combinations.

This study aimed to evaluate acute aquatic ecotoxicity of four synergistic NC-ATB combinations compared to individual compounds, identifying synergistic toxic effects on non-target organisms. The tested combinations, previously validated against human bacteria, were cinnamaldehyde (CIN) with amoxicillin (AMO), CIN with gentamicin (GTM), eugenol (EUG) with GTM, and EUG with tetracycline (TC). Ecotoxicity assays were carried out using three bioindicators: luminescence inhibition of *Aliivibrio fischeri*, immobilization of *Daphnia magna*, and community-level physiological profiling (CLPP) via Biolog AWCD on river microbiota. Tests were performed at Minimum Inhibitory Concentrations of isolated (MIC_{iso}) and combined components (MIC_{comb}). Results showed synergistic combinations generally had higher ecotoxicity than isolated ATBs on *A. fischeri* and *D. magna*. For example, the CIN-AMO combination's *D. magna* EC₅₀ was 16.191 mg/L, markedly lower than individual components at similar doses. Conversely, toxicity toward aquatic microbiota was comparable to isolated ATBs at MIC_{iso} and lower than isolated NCs at MIC_{iso}. Notably, EUG-TC was the most toxic to river microbiota, reducing AWCD almost to zero, implying a strong impact on bacterial metabolism. Differences between toxicity in simple organisms and complex microbiota suggest toxic effects could be masked in natural communities due to resistant bacterial species proliferation, consistent with findings in other chemical combination studies.

Though environmental concentrations are low compared to EC₅₀ values, NC-ATB synergies are not ecologically innocuous for selected non-targets, showing unexpectedly enhanced toxicity. The substantial ecotoxicity increase over individual ATBs, especially for *A. fischeri* and *D. magna*, is a critical anomalous challenge that current risk assessment models do not consider because those assume low risk from NCs and focus on isolated antibiotics. Future environmental impact assessments of novel antimicrobial strategies must consider synergistic effects.

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3.21.P-Tu365 Ecotoxicological Effects of a Thymol–Chloramphenicol Synergistic Antimicrobial Combination on *Daphnia magna* and River Microbial Communities

Cristina Gan-Oria, Elisa Langa, Laura Botello-Morte, Diego Ballesterio and María Rosa Pino Otín, San Jorge University, Spain

The extensive use of antibiotics has led to their persistent release into aquatic environments, raising concerns about resistance development and toxicity to non-target organisms. Combining natural compounds with conventional antibiotics is emerging as a sustainable approach to reduce antibiotic doses while retaining antimicrobial effectiveness. However, the environmental consequences of such synergistic antimicrobial combinations (SACs) remain poorly characterized. This study investigated the ecotoxicity of a thymol–chloramphenicol SAC previously shown to exert potent antimicrobial activity against *Acinetobacter baumannii* but whose effects on aquatic organisms and microbial communities had not been examined.

Acute immobilization assays with *Daphnia magna* were conducted according to OECD Guideline 202, using five dilution levels (100%, 50%, 25%, 12.5%, and 6.25%) of the SAC. The undiluted mixture contained 31.25

µg/mL thymol and 7.8 µg/mL chloramphenicol. Immobilization increased with concentration and exposure time, with 48-hour EC50 values indicating moderate acute toxicity. Predictive mixture models (Concentration Addition and Independent Action) revealed that the observed toxicity closely matched the expected response derived from the individual substances, suggesting no synergistic or antagonistic effects on *D. magna*. To assess potential impacts on microbial communities, water from the Gállego River (Spain) was exposed to 31.25 µg/mL thymol, 7.8 µg/mL chloramphenicol, their combination (SAC), and the individual compounds at their respective minimum inhibitory concentrations (MICs; thymol 125 µg/mL, chloramphenicol 62.5 µg/mL). Microbial metabolic activity was evaluated with Biolog EcoPlate™, assessing the ability of native microbial assemblages to utilize 31 carbon sources. Significant reductions in average well color development (AWCD) were observed at MIC levels of the individual compounds, showing stronger inhibition than that produced by the SAC. At synergy concentrations, both thymol and chloramphenicol exhibited much lower toxicity, and the thymol response was similar to the negative control. Overall, the thymol–chloramphenicol combination preserved potent antimicrobial activity while markedly reducing the individual compounds' toxicity. These findings support the potential of natural–antibiotic synergistic formulations as environmentally safer alternatives to mitigate the ecological impact of antibiotic usage

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3.21.P-Tu366 Ecotoxicological Assessment of Synergistic Antimicrobial Combinations of Antibiotics with Tannic Acid (SACTA) and Nerol (SACNE) on Aquatic Bioindicators and River Microbial Communities *Guillermo Lorca, Diego Ballester, Laura Botello-Morte, Pedro Rodríguez-López, Elisa Langa and María Rosa Pino Otín, San Jorge University, Spain*

Synergistic combinations between antibiotics (ATB) and natural products (NP), such as tannic acid (TA) and nerol (NE), are promising strategies to reduce antibiotic doses and mitigate antimicrobial resistance and environmental contamination. However, their ecotoxicological impacts on aquatic systems and microbial communities remain poorly understood.

This study assessed six synergistic combinations: SACTA1 (TA 46.87 + chloramphenicol, CHL 3.91 mg/L), SACTA2 (TA 46.87 + ampicillin, AMP 7.81 mg/L), SACTA3 (TA 40.62 + CHL 1 mg/L), SACNE1 (NE 125 + streptomycin, STM 1.17 mg/L), SACNE2 (NE 125 + gentamicin, GTM 0.78 mg/L), and SACNE3 (NE 250 + STM 31.25 mg/L), using *Daphnia magna*, *Aliivibrio fischeri*, and microbial assemblages from the Gállego River (Spain).

For *D. magna*, toxicity decreased in the order SACNE3 > SACNE2 > SACNE1 > SACTA3 > SACTA2 > SACTA1; for *A. fischeri*, SACNE2 > SACNE3 > SACNE1 > SACTA2 > SACTA1 > SACTA3, confirming higher toxicity of NE-based mixtures. Antibiotic EC50 values in combinations (0.03–5.47 mg/L) were 100–10,000 times lower than their individual LC50 values (STM 772, GTM 840, AMP 4324, CHL 285 mg/L), demonstrating the co-adjuvant effect of NPs. Despite increased mixture potency, antibiotic concentrations remained below therapeutic MICs, ensuring environmental safety. NP doses (NE 1–14.6 mg/L; TA 18.12–43.17 mg/L) were below or near LC50 levels. Overall, synergistic use reduced antibiotic requirements by up to 90%, decreasing selective pressure and resistance risk.

At the community level, TA-based mixtures (SACTAs) reduced microbial growth and degradation of polymers and carbohydrates, whereas NE-based (SACNEs) caused milder effects, with SACNE1 eliciting a hormetic response enhancing growth and amino acid metabolism. Higher TA toxicity reflected disruption of ecological and metabolic interactions, while NE had direct membranolytic effects. SACNE1, SACNE3, SACTA1, and SACTA2 were 16–32% less inhibitory than individual antibiotics at MIC, SACNE2 matched antibiotic toxicity, and SACTA3 was 71% more toxic.

These findings show that ecotoxicity of natural–antibiotic synergies depends on composition. The limited impact and resilience of microbial communities support their potential as safer antimicrobial alternatives, inviting further validation in realistic environmental scenarios.

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3.21.P-Tu367 A Cocktail a Day Keeps the Doctor Away - How to Consider Potential Additive Mixture Toxicology within Veterinary Pharmaceutical Legislation

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Conventional ecotoxicological assessments typically focus on assessment of single substances or of fixed mixtures in combination products. Those are mostly ignoring and thus underestimating the effects of additive or synergistic mixture toxicology in the environment. Among others, this is true in the context of the assessment of pharmaceuticals. Therefore, this study assesses how a Mixture Assessment Factor (MAF), based on data for veterinary pharmaceuticals might statistically be defined, considering the extensive variety of chemical mixtures possible, and what role single substance tests and measured environmental concentrations (MECs) can play in this process.

The proposed approach includes publicly available databases, such as the Pharms-UBA (PU) for MECs and the European Medicines Agency's public assessment reports for effect data. The latter were supplemented by internal data from the German Environment Agency. Based on that dataset, four questions were posed: (1) Which mixtures are found in the environment, and in what range are the additive MECs? (2) Are there any substances and mixtures that raise concerns due to potential $RQ_{real} = MEC/PNEC$ (real world RQs)? (3) Which MAF_{max}, MAF_{weighted}, and MAF_{exact} can be derived? (3) Based on additive effects, what would be a generally applicable MAF suggest?

The effect dataset contains 51 AS, which can theoretically form 4.22×10^{66} mixtures. Thus, the most relevant mixtures need to be found. The PU MEC database shows that, only based on soil and veterinary pharmaceuticals, 34 different AS can be found with theoretical 8.03×10^{38} mixtures possible. The articles contained 219 study sites where mixtures actually occurred in the environment. Mixture sizes ranged from 2 to 17 with additive MECs from ng up to mg/kg soil.

In summary, our analysis demonstrates that mixtures in the environment already are a cause for concern and should be addressed in Environmental Risk Assessment. Based on veterinary and dual-use AS, it highlights the need for an extra safety factor due to already elevated mixture exposure. This suggests a similar approach for human pharmaceuticals, identifying relevant mixtures, risk drivers, their occurrence, risk potential, and possible regulatory adaptations to align with environmental protection goals.

Disclaimer/Disclosure:

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3.21.P-Tu368 The Missing Pieces for a Monography System – Prioritizing Substances for Pharmaceutical Environmental Risk Assessment

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In order to minimize risks posed by pharmaceutical residues in the environment, it is necessary to understand the potential effects on organisms and the fate of these substances in the environment. Especially for substances authorized before 2005 for use in veterinary medicinal products (VMP) and in 2006 for use in human medicinal products (HMP) respectively, such data are often missing. Even for products approved after the relevant guidelines come into effect, data gaps are quite common. In 25 % of substances authorized, no final assessment is possible. Incomplete dossiers due to tests with insufficient data quality further reduce the ability to estimate the potential environmental impact. In Article 23 of the proposed revision of Directive 83/2001, a program for legacy medicinal products authorized before 2005 is planned. Therefore, a prioritization scheme will be needed to unveil the most important substances connected to eventually occurring adverse environmental effects. For that, publicly available data from (European) Public Assessment Reports will be supplemented by confidential data from the German Environment Agency. Predicted Environmental Concentrations will be generated and supplemented with available refined PECs. The analysis starts with a holistic approach, where

active substances of both HMPs and VMPs are examined with respect to their main entry pathways, regardless their indication or anatomical therapeutically group. For calculations including MECs, among others, the Pharms-UBA-Database will be used.

Following an evaluation of the criteria, the final step will be the creation of a prioritisation scheme for such a programme. This will include a list of active substances that are of environmental concern and/or for which data is missing. It will also highlight the amount of already available data, where generation of the proposed substance monographs (Article 24 of proposed new Directive) is a low-hanging fruit. The objective is building a strong data foundation for adequate risk assessment in the regulation and use of pharmaceuticals. This helps to avoid redundant work in substance evaluations, facilitates product substitution and enables the implementation of safety precautions during production and use. Combining such a foundation in form of a monograph system and explicit data-sharing guidelines aids process simplification in preventing unnecessary multiple assessment and mitigates resource consumption for all stakeholders involved.

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3.21.P-Tu369 Application of Toxicogenomics in Ecotoxicological Model Organisms for Monitoring Pharmaceutical Residues as Relevant Trace Compounds in Aquatic Environments

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Relevant trace substances, such as nanoparticles, per- and polyfluoroalkyl substances (PFAS) and pharmaceuticals, enter wastewater systems through domestic wastewater, industrial discharges, or rainwater and, if not efficiently removed, can contaminate rivers, lakes and even drinking water. Pharmaceuticals as highly potent substances pose a significant threat to aquatic compartments and their communities. The effects on aquatic organisms range from acute to chronic toxicity, sometimes even at environmentally relevant concentrations. Moreover, pharmaceuticals often occur as complex mixtures whose combined effects may exceed those of their individual components. A major challenge in monitoring pharmaceuticals in aquatic systems is their presence at very low concentrations, which makes analytical detection difficult. Targeted analytical methods are limited to known compounds, whereas non-targeted approaches often lack sensitivity, especially for compounds in complex mixtures. Detecting transformation products is particularly challenging, as little data is available on them.

The aim of this study is to investigate the applicability of toxicogenomic methods for detecting the molecular effects of pharmaceuticals as trace substances in aquatic environments. Based on this, a mechanism-sensitive monitoring approach will be developed and applied to pharmaceutical mixtures and real water samples to detect compounds by their mode of action (MoA).

Relevant OECD guideline tests will be combined with transcriptomic methods using the aquatic model organisms *Danio rerio* (embryo) and *Raphidocelis subcapitata*. Single substances as well as simple and complex mixtures will be tested, starting with preliminary tests to determine sublethal effect concentrations, followed by testing down to the environmentally relevant concentration range. Transcriptome analysis, transcriptomic-point-of-departure (tPOD) calculation and bioinformatic evaluation will be used to investigate method sensitivity and identify molecular signatures of the MoA of the tested pharmaceuticals. This approach will then be applied to mixtures and water samples, such as effluents from wastewater treatment plants.

Overall, the results of this study could enable the applicability of molecular methods for monitoring pharmaceuticals in environmental samples to be investigated, supporting both environmental monitoring and risk assessment.

3.21.P-Tu370 A Novel Secondary Poisoning Model for Pharmaceutical Bioaccumulation in Aquatic, Terrestrial, and Hybrid Food Webs

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Pharmaceutical pollution has become a salient issue in recent years, where contamination is not only limited to the aquatic environment, but also terrestrial and subterranean environments. However, due to the scarcity of data when compared to the large number of pharmaceuticals and biota that can be affected, secondary poisoning models can be deployed to fill in these data gaps both in terrestrial and aquatic environments. Yet many models have limited applicability, either by their limited species scope or by not considering physicochemical properties such as ionisation, which is specifically important for pharmaceuticals. Therefore, a novel secondary poisoning model would need to account for both the complexity and diversity of the ecosystem it is modelling, and the diversity of physicochemical properties that pharmaceuticals particularly may have. To this end, a new secondary poisoning model was developed and validated that covers the aquatic, terrestrial, and subterranean food webs, as well as combinations.

The model expands on previous models by including ionisation and ionisation-based phase partitioning using logDOW and poly-parameter linear free energy relationships (pp-LFERs). The model covers multiple species groupings (algae, plants, worms, molluscs, arthropods, fish, birds, and mammals) across three environments (aquatic, subterranean, and terrestrial) while correcting for mass, blood type, diet, and internal pH. The model was validated using 75 pharmaceuticals spanning 55 studies, 330 compounds, and 445 species from recent literature. Fold errors showed that 81% of aquatic data had been within a factor of 100, while 50% was within a factor of 10. Preliminary results for the terrestrial model indicate that 71% of predicted data were within a factor of 100, while 51% were within a factor of 10. Algae, was consistently under predicted by the model, indicating that cell wall interactions may need to be modelled differently. Individual compounds such as caffeine and sulfamethoxazole also routinely were underpredicted, with sulfamethoxazole being a prime example of a compound with multiple ion species and zwitterions, which the model does not account for. Furthermore, terrestrial data were only limited to 3 studies, indicating that improvements to the terrestrial and subterranean parts of the model could be made with the availability of more data.

3.21.P-Tu371 K drugs: Eco/genotoxicological Characterization of the synthetic Cannabinoid MDMB-4en-PINACA

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The use of chemical substances to alter mental states and sensory perception dates back centuries. New Psychoactive Substances (NPS) are compounds designed to modulate the central nervous system. Synthetic cannabinoids (SC), popularly known as “K drugs” (K2, K4, K9), represent the group with the highest number of substances detected worldwide since 2008. MDMB-4en-PINACA stands out for its strong affinity to CB1 receptors in the central nervous system. Data on the ecotoxicity, genotoxicity, and behavioral effects of NPS remain scarce, limiting our understanding of their environmental impacts. This work aims to characterize the eco/genotoxicological hazards of the synthetic cannabinoid MDMB-4en-PINACA. MDMB-4en-PINACA was dissolved in Dimethylsulfoxide (DMSO) at the limit of solubility (11.26 µg/µL). Acute toxicity was performed with *Daphnia similis* (2 to 11 mg/L) and *Parhyale hawaiiensis* (2 to 11 mg/L). A miniaturized version of the Salmonella/microsome mutagenicity test (Microplate agar, MPA) was tested in concentration-response from 0.14 to 2.25 µg/µL. Five strains (TA100, TA98, TA97a, TA102, and TA1535) were used with and without metabolic activation (rat liver S9 5% PB-induced). DNA damage in *P. hawaiiensis* sperm cells was evaluated using the comet assay at three concentrations (0.5, 1.6, 5.0 mg/L), along with negative (DMSO 0.01%) and positive controls (Ethyl methanesulfonate, EMS). Sixteen males were individually exposed for 96h. Testes were collected, slides prepared and stained with ethidium bromide. One hundred comets per gel were analyzed (%DNA in tail) using Comet Score 2.0. Acute toxicity was observed in both organisms, *D. similis* (EC50 3.77 mg/L) and *P. hawaiiensis* (LC50 2.91 mg/L). MDMB-4en-PINACA did not showed mutagenic effects in any Salmonella strain, regardless metabolic activation, but increased sperm DNA damage at 5.00 mg/L. This work provides new data on the eco/genotoxic hazard characterization of the synthetic cannabinoid MDMB-4en-PINACA. *P. hawaiiensis* proved to be a suitable invertebrate model for assessing sperm DNA damage after 96 h exposure to MDMB-4en-PINACA. Using invertebrates as alternative models helps reduce ethical and social concerns associated with mammalian testing. To complete the mutagenic evaluation, we are testing it for micronucleus in hemocytes of *P. hawaiiensis*.

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3.21.P-Tu372 Environmental Risk Assessment of Generic Human Pharmaceuticals

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Following adoption of the updated environmental risk assessment (ERA) guidelines by the European Medicines Agency (EMA) the assessment of generic human pharmaceuticals is entering a new era of scrutiny. Both the EMA and the UK Medicines and Healthcare products Regulatory Agency (MHRA) are increasingly requiring full environmental data packages for generic pharmaceutical applications, an evolution that reflects the growing priority placed on environmental protection under EU and UK frameworks.

Historically applications for generic products could avoid in-depth ERAs by arguing that authorisation would not lead to increased environmental exposure. However, this approach is now only accepted under the new guideline if a full ERA is available for the reference drug product. In cases where no previous ERA is available, e.g. original submission occurred before 2006, or the previous ERA does not meet the requirements of the current guidelines, new or updated ERAs will need to be submitted.

This shift aligns with broader environmental objectives across the EU and UK, ensuring that all pharmaceuticals are assessed for their potential impact on ecosystems, consistent with guidance under Directive 2001/83/EC and UK Human Medicines Regulations 2012.

With the adoption of the updated EMA guidelines many generic pharmaceutical manufacturers find themselves requiring extensive ERAs, including significantly laboratory testing, to fulfill their submission requirements. For generic manufacturers margins are tight and the requirement for laboratory testing often exceeding €300,000 may make some products financially unviable. The aim of this work is to discuss the strategies available to manufacturers of generic pharmaceuticals to satisfy the requirements of the EMA guidelines and highlight the benefits of data sharing and collaboration during data generation.

Overall, the implementation of the mandatory environmental risk assessment for pharmaceuticals products is leading to a strong increase of new experimental studies for complex endpoints. To avoid unnecessary animal testing and ensure harmonization of ERAs on the same active substances, generic manufacturers must be aware of the options available and the benefits of collaboration through development of consortium and data sharing agreements.

3.21.P-Tu373 Should Peptide-Based Radioligand Therapeutics be Exempt from an Environmental Risk Assessment under the EMA Guideline for Human Medicinal Products?

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A radioligand therapy is a precision-based approach for cancer treatment. Peptide-based radioligands include a peptide-based targeting compound, a linker, and a complexing agent to chelate a radioactive isotope. The ligand binds to a specific cell marker of the target cancer cells to deliver the radioisotope, destroying these cancer cells while having limiting impact on nearby healthy cells. The radioisotopes used have short decay half-lives (e.g., in the order of a few hours to a few days) to minimize long-term exposure.

Risks related to the radioactive properties of these therapies are governed by separate regulatory frameworks. In addition, the EMA guideline (EMA/CHMP/SWP/4447/00 Rev. 1- Corr.*), does not exempt radioligands from an environmental risk assessment. The assessment starts by estimating predicted exposure concentration (PEC) and determining n-octanol:water partitioning coefficients (log Dow) at environmentally relevant pHs.

The therapeutic doses are defined based on radioactivity rather than mass. As a worst-case scenario, the maximum mass content of the chemical precursor (typically at levels of µg per dose) can be used in the PEC calculation. The resulting PEC, considering also the dose regimen and the default market penetration factor (1% of the population per year) leads to PECs several orders of magnitude below 0.01 µg/L, the phase II ERA threshold. Additionally, if the PEC was refined by prevalence of the target indications, it would be even lower. The log Dow values are to be compared with a threshold of log Kow > 4.5, as a trigger for a persistence-bioaccumulation-toxicity assessment. Peptide-based radioligands have predicted log Kow values in the negative range, driven by both the peptide and the chelator hydrophilicity. Handling of the radioligand to generate experimental log Dow data would be technically challenging and unnecessary given the hydrophilicity of the radioligand.

Given 1) the extremely low predicted environmental exposure, 2) the short decay half-life of radioligands and consequent rapid loss of pharmacological activity, 3) the fact that a large fraction of their molecular mass corresponds to the peptide, which is expected to readily biodegrade, and 4) the high hydrophilicity of peptide-based radioligands, we consider that peptide-based radioligands should be exempted from the preparation of an environmental risk assessment.

3.21.P-Tu374 Environmental Risk Assessment of the New Pharmaceutical Imlunestrant, a Selective Estrogen Receptor Degradar

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Imlunestrant is a novel, small molecule estrogen receptor degrader that has recently been approved to treat breast cancer. To fulfill the Environmental Risk Assessment (ERA) requirement, studies to characterize the physical chemical properties, environmental fate and ecotoxicity of imlunestrant were conducted. The results of these studies were used to predict the distribution, persistence and effects of imlunestrant amongst environmental compartments. A fish full- life cycle toxicity test was included based on the mechanism of action at the estrogen receptor. A high adsorption coefficient to sewage sludge ($K_{FOC} = 29600 \text{ L}\cdot\text{kg}^{-1}$) indicates that imlunestrant could be transferred to soil via land application of biosolids; therefore, the toxicity to terrestrial species was evaluated. Imlunestrant is hydrophobic (at pH 5, 7, 9 $\log K_{ow}$ is 3.29, 5.13, 5.55, respectively) so a bioconcentration study in fish was conducted. Risk assessments were conducted for the sewage treatment plant, surface water, sediment and soil. Risk Quotients (PEC:PNEC) were all less than 1 and demonstrated that there is no risk to species in the environment. While the BCF_{kgL} was determined to be 274 to 500 $\text{L}\cdot\text{kg}^{-1}$, preclinical toxicity data was evaluated to show that imlunestrant does not pose a risk for secondary poisoning. Additionally, imlunestrant is not classified as PBT or vPvB. Conduct of the ERA followed the framework of the updated EMA ERA guidance (2024) and learnings with the new guidance are discussed.

3.21.P-Tu375 Environmental Risk assessment of Pharmaceuticals in river catchment areas – case study of Latvia

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Pharmaceuticals are the backbone of therapeutic medicine, improving quality of life and extending life expectancy worldwide. However, there is growing concern about their environmental impacts—both during manufacturing and after the post-consumption excretion of the more than 4,000 unique active ingredients currently listed by DrugBank. While the EU has established several frameworks and directives to support the removal and monitoring of micropollutants (including pharmaceuticals), there remains no harmonized or clearly defined approach for evaluating environmental risks beyond individual substance assessments. To address this mixture-based challenge, we propose a cumulative risk-calculation approach based on risk-quotient (RQ) aggregation with adjusted logistic function in a descriptor the authors coined component cumulative risk index (CCRI). The CCRI provides a more scaled estimate of mixture risk than the simple sum of RQs. While sum of RQ increases linearly and can therefore overestimate risk when many substances are present at low levels, the CCRI moderates this inflation and is interpretable across different sites and seasons.

To test the robustness and practical relevance of this approach, we analyse a case study using real measured concentrations of 10 pharmaceuticals in Latvian surface across all four seasons.

A seasonal trend was evident throughout study period with the concentrations decreasing from winter to summer. The overall RQ-sum values were highest during November 2024, moderately elevated in February 2025, and mostly lower in May 2025. Natural waterbodies—upstream sampling sites, reservoirs, and river mouths—consistently exhibited low risk values, typically below an RQ of 1. Downstream sites receiving WWTP discharge showed higher RQ sums than natural waters, but these values were still substantially lower than those measured directly in WWTP effluents, which were often one to two orders of magnitude greater. This trend was also reflected in the cumulative risk assessment based on CCRI, which showed that a small subset of pharmaceuticals—diclofenac, clarithromycin, estrone, sulfamethoxazole, and venlafaxine—consistently dominated the total RQ contribution across sites and seasons.

The results highlight the importance of mixture-based evaluation such as the CCRI, showing that a small number of high-impact pharmaceuticals can disproportionately shape environmental risks and should be prioritized in monitoring and management strategies.

Disclaimer/Disclosure:

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3.21.P-Tu376 Addressing Public Data Deficiency: Evaluation of AI-Tool "TRIDENT" for the Generation of Ecotoxicological Data in Context of Pharmaceutical Environmental Risk Assessment (Art. 57(2) EC

No. 726/2004)

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Medicines that were approved for the EU market before 2005 were not required to undergo an environmental risk assessment (ERA) as part of the EU approval process. Even with the current European legislation, data that is collected remains not sufficiently accessible to the public. This results in a data gap as described by Spilsbury et al. (2024), among others. This has led scientists to explore other methods to assess the environmental impact of the active pharmaceutical ingredients (APIs) in medical products able to help close the knowledge gap. The emergence of AI models offers new opportunities for in silico modelling of ecotoxicological endpoints. This study uses the AI-based tool TRIDENT, which, according to Gustavsson et al. (2024), delivers more reliable results and has a broader range of applications than conventional in silico methods based on quantitative structure-activity relationships. This study modelled and validated predicted ERA endpoints for the aquatic toxicity of environmentally relevant pharmaceuticals approved for sale in the EU in accordance with Article 57(2) of Regulation (EC) No. 762/2004. A possible assessment factor is determined by comparing the modelled results from TRIDENT with available empirical data. APIs were then prioritized based on these results and compared with existing lists of priority substances. Furthermore, the underlying training data is evaluated to determine which factors are critical for more reliable prediction data prioritization efforts for pharmaceuticals.

3.21.P-Tu377 Too Safe for Comfort? Examining the Conservatism in Sediment Risk Assessment of APIs

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The EMA Phase 2 sediment environmental risk assessment derives predicted sediment concentrations (PEC_{sediment}) from surface water predictions (PEC_{surface water}) using an equilibrium partitioning method (EPM) at Tier A. Tier B refinements address only the PEC_{surface water} input and no guidance is provided for incorporating degradation in water or sediment or adsorption-desorption kinetics to improve the realism of sediment exposure prediction. Using AstraZeneca's EcoPharmacoVigilance dataset, approximately 200 matched literature pairs of measured concentrations in surface water (MEC_{surface water}) and sediment (MEC_{sediment}) were compiled. MEC_{surface water} values were used to derive, for comparison PEC_{sediment} according to EMA ERA. EPM-based PEC_{sediment} systematically exceeded MEC_{sediment}, demonstrating added conservatism beyond the already conservative PEC_{surface water} under the current sediment RA approach. For the three most data-rich APIs, the predicted-to-measured ratios were high: "API A" averaged a 48-fold overestimate; "API B" averaged a 300-fold overestimate; and "API C" averaged a 2-fold overestimate, with 9 of 49 pairs for "API C" only (and 9 of 177 pairs in total) showing MEC_{sediment} higher than PEC_{sediment} (all underpredictions being less than fivefold). These findings indicate the current approach necessarily overestimates sediment organism exposure for Phase 2 APIs that undergo any degradation in water, sediment, or both, potentially flagging unwarranted sediment risks. With anticipated GPL revisions that may tie environmental risk to approval timelines or mitigation requirements, relying on the unrefined EPM may lead to unnecessary mitigations. Incorporating available water-sediment degradation data (e.g., DT₅₀ values) from OECD water-sediment tests offers potential refinement options to differentiate risks driven by overly conservative assumptions from genuine potential risks and improve the realism and regulatory relevance of sediment exposure predictions.

3.21.P-Tu378 A Scientifically Robust and Regulatory Critical Fish Full Life Cycle Study with Ibuprofen: Fulfilling Chronic Toxicity Data Gaps to Support Informed Environmental Risk Assessment

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Robust chronic ecotoxicological data are critical for pharmaceutical registration within the EU, as outlined by the European Medicines Agency (2024), and plays a pivotal role in policy development and regulatory decision-making. The Environmental Quality Standard (EQS) dossier SCHEER (2023) and Faber et al. (2023) highlights that although several freshwater toxicity studies on ibuprofen exist, many fail to meet CRED scoring criteria, resulting in notable gaps and uncertainties when assessing and drawing conclusions on the chronic aquatic toxicity profile of Ibuprofen. Oldenkamp et al. (2022) similarly stressed the importance of high-quality, accessible ecotoxicity data on pharmaceuticals to support scientific research, regulatory frameworks, and public awareness.

Due to the considerable time, complexity and expenditure involved in conducting fish full life cycle tests, most current fish toxicity studies such as Constantine et al. (2020) focus primarily on early life-stage development or sensitive developmental stages in isolation rather than considering the reproductive output across generations which could prove more relevant for understanding population level effects and support well informed environmental risk assessments when scientifically appropriate.

The authors used a collaborative approach that included consultation with leading academic experts and regulatory authorities to develop a full life cycle test suitable for investigating the toxicological effects of Ibuprofen to fish (*Pimephales promelas*) with the intention to continuously expose F0 embryos (<24-hours old) through all growth and reproduction stages to the production and growth of F1 juveniles.

This test represents the first full life cycle test conducted with ibuprofen in accordance with Good laboratory Practice (GLP). This study holds high significance and provides the most sensitive aquatic endpoint from a comprehensive series of environmental toxicology tests conducted by the authors between 2019 and 2025. This test provides robust endpoints considered critical for understanding effects of ibuprofen to fish and enables well informed environmental risk assessments. This study builds on earlier work published by Constantine et al. (2020), and presents the first scientifically relevant and reliable Fish Full Life Cycle Study with ibuprofen (CRED C1, R1; Moermond et al., 2016).

3.22 PMT/vPvM Substances: Advances in Identification, Prioritisation, Risk Assessment and Regulation

3.22.T-01 Percentage Leached as a Function of Koc and DT50 – a Scientific Indication of True Mobility *Sevil Payvandi¹, Siul Ruiz², Jose Luis Gomez-Eyles¹, Paul Sweeney¹ and Tiina Roose², (1)Syngenta, United Kingdom, (2)University of Southampton, United Kingdom*

Many factors are involved in the leaching of substances to groundwater, e.g. substance properties, emission at the soil surface, and environmental conditions. Of the substance properties, the degradation half-life and sorption partition coefficient are the most important, and represent key inputs into regulatory environmental risk assessments as well as many empirical indices of leaching.

In terms of assessing hazards (as opposed to assessing risk), mobility in soil represents the hazard of leaching, where mobility has been defined by REACH Annex II, as ‘the potential of the substance...to move under natural forces to the groundwater or to a distance from the site of release’. In Europe, the organic carbon-water partition coefficient (Koc) alone has been proposed as the basis of the mobility hazard classification. However, whilst Koc is clearly important for leaching, this approach ignores the other critical substance property that governs true mobility and leachability – the soil degradation half-life (DT50). Here we present how a simple ratio of sorption and degradation can be used to provide a more robust hazard assessment of mobility based on substance properties.

We extend previous work where leaching concentrations were estimated analytically. In that work, to enable analytical progress, we considered the steady state solution of the leaching concentration as a function of depth under constant effective water flux conditions and used European FOCUS scenarios. Variability of weather conditions was accounted for by sampling over the weather files. To now address the estimation of percentage leached, we adapt the previous work and as an example we consider all European FOCUS scenarios. However this method could be rapidly applied to any representative region or a combination of regions as the solutions are analytical. We then derive critical thresholds of Koc/DT50 and analyse how these could be used as hazard criteria.

The ratio of Koc/DT50 represents a simple but scientific extension of the hazard concept of mobility by combining half-life and sorption while still retaining the conservatism of the original concept of using sorption alone, and the extent of leaching is considered and not just the extent of sorption. In addition, we will also address how to use this methodology for higher tier refinements and weight-of-evidence cases e.g. modelling approaches to integrate aged sorption data into mobility hazard assessments.

3.22.T-02 Advancing Multi-Model Screening of Persistent, Mobile and Toxic Chemicals: Application to the DSSTox Database

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Persistent, Mobile, and Toxic (PMT) substances, as well as very Persistent and very Mobile (vPvM) substances can cause long-lasting and diffuse contamination of water resources. To facilitate early identification and prioritization for regulatory action, the Dutch National Institute for Public Health and the Environment (RIVM) developed a novel screening method in 2023, initially applied to over 6,000 chemicals. Each substance receives a continuous score (0–1) for persistence (P), mobility (M), and (human) toxicity (T), which are aggregated into an overall PMT score. These data are made accessible to permit officers, risk assessors, water suppliers, industry, and other stakeholders via an RIVM-hosted website.

Currently, RIVM is refining and expanding this approach based on stakeholder feedback and recent scientific insights. Key improvements include: extending the method to the Distributed Structure-Searchable Toxicity (DSSTox) Database (>1 million substances), incorporating a broader set of models for P, M, and T estimation, and explicitly addressing uncertainties. Furthermore, RIVM is moving the workflow from Excel to R and Python, enhancing reproducibility, scalability, and transparency, and enabling on-demand model execution.

As the revised method is still in development, results are pending. The presentation will focus on methodological improvements and application to the DSSTox database. Comparisons with known and suspected PMT-substances from priority lists and monitoring programs from drinking water companies will also be presented.

The improved screening approach and expanded database increase usability for regulators and stakeholders in identifying and managing environmentally relevant chemicals. This tool serves as an initial step, guiding further investigation of high-scoring substances and supporting safe-by-design strategies to prevent the development of new PMT chemicals.

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3.22.T-03 Prioritization of PMT Chemicals during Agricultural Water Reuse – Potential for Human Exposure and Groundwater Contamination

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With increasing water stress agricultural water reuse is becoming more prevalent 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 the water cycle. If these chemicals are also toxic (T), they may pose a threat to human and environmental health. Consequently, these PM(T) chemicals seem highly relevant for water reuse, yet little is known on their up-take by and distribution in plants or infiltration into groundwater. In this study, two exposure pathways during agricultural wastewater reuse are studied for 60 PM(T) chemicals: (I) the potential for human exposure through crop consumption is investigated by studying plant uptake by and distribution in rocket in pot experiments in a greenhouse, and (II) infiltration into groundwater is simulated with soil-filled columns over a duration of 37 weeks. Combined, these results aid in prioritizing potentially problematic PM(T) chemicals during agricultural water reuse and demonstrate which exposure pathway is most relevant for these chemicals.

The relative uptake (% RU – fraction of maximum uptake over experiment duration) by rocket exceeded 10% RU for 18 PM(T) chemicals and reached up to 64% RU for perfluoropropionic acid (PFPrA). Carbamazepine, a well-studied pharmaceutical known for its very high plant uptake showed a plant uptake of 28% RU. To investigate the distribution of PM(T) chemicals in arugula the translocation factor was calculated. More than 60% of the studied chemicals showed a translocation factor above 1, which indicates accumulation in the rocket leaves with individual chemicals reaching a translocation factor of up to 100.

A total of 87% of the target compounds were detected in the column effluent. The results showed that 23 analytes experienced virtually no retardation (retardation coefficient $RD < 1.5$ relative to bromide tracer, Figure

1), 6 analytes exhibited moderate retardation (RD 1.5–5), and 28 PM chemicals eluted markedly delayed (RD > 5). Ultrashort-chain perfluoroalkyl substances, such as PFPrA, trifluoroacetic acid, and bistriflimide, broke through rapidly and were detected in the effluent at more than 40% of the applied mass. Based on these findings and median concentrations in urban effluent a human exposure potential and a ground water exposure potential were calculated aid in prioritization for risk assessment.

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3.22.T-04 Identifying Persistent and Mobile Substances with the SIN List

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The Substitute It Now! (SIN) List is an early-warning phase-out list of chemicals that, according to the non-governmental organisation ChemSec, meet the regulation on the registration, evaluation, authorisation and restriction of chemicals (REACH) criteria for substances of very high concern (SVHC). In October 2025, the SIN List was updated with 18 new persistent, mobile and toxic (PMT) and very persistent and very mobile (vPvM) entries as part of the Zero Pollution of Persistent, Mobile Substances (ZeroPM) project.

Persistent and mobile substances threaten water resources, including drinking water, as their continued release leads to an increase in contamination. In 2023, the Classification, Labelling and Packaging (CLP) regulation introduced PMT/vPvM as new hazard classes. This development represents an important initial step towards acknowledging the urgency of regulating chemicals that contribute to water resource contamination. The current inclusion of 53 PMT/vPvM entries on the SIN List further substantiates this urgency and provides industry and other stakeholders with a relevant tool for chemical substitution.

The PMT/vPvM substances were assessed through a weight-of-evidence approach based on the CLP criteria. Data were retrieved from REACH registration dossiers, other authorities' publicly available sources, and scientific literature.

The new SIN List entries have a variety of characteristics, both in terms of chemical groups (mainly nitrogen compounds, but also halogenated, aromatic and heterocyclic compounds, and hydrazines), and production volumes (from ? 1 to < 10 tonnes per annum for dichlorodifluoromethane, to exceeding 10 000 tonnes per annum for 1,3-dioxolane, acryloyldimethyltaurates (AMPSA), cyanoguanidine, cyanuric chloride and methenamine). The substances are in both industrial and consumer use. Some have very specific technical functions (i.e. benzotriazoles and methylated benzotriazoles are anti-corrosion agents) while others are more versatile and can have multiple functions (e.g. methenamine, cyanoguanidine).

The full list of PMT/vPvM entries can be found on the SIN List (<https://sinsearch.chemsec.org/>).

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3.22.T-05 Protecting Drinking Water Sources from PMT-substances

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Protection of drinking water sources is part of the so-called Multi-Barrier Approach To Safe Drinking Water. One important barrier is source water protection, which may sound obvious but can get very complicated in a densely populated area with multiple uses such as the Meuse river basin. Drinking water companies extract around 460 million cubic metres of water from the Meuse every year to supply drinking water to around 7 million people in the Netherlands and Belgium.

One of the components of source water protection is monitoring and for compliance checks monitoring results need to be compared to norms or standards set by legislation. But not all substances that can be monitored have legal standards set. This is specifically the case for a wide variety of persistent, mobile and toxic substances which are hard to remove with most regular water treatment techniques. Therefore 170 drinking water companies from 18 EU countries have set target values for unregulated parameters in the European River Memorandum.

The drinking water companies that use the river Meuse as a source for drinking water production monitor several hundreds of parameters. Every three years the monitoring results are evaluated against the ERM target values. The latest evaluation was done in 2024. More than 282,000 measurements were evaluated based on concentration, frequency of detection and intrinsic properties that hinder sustainable production of drinking water. In total 37 substances were prioritised as relevant, meaning that they hinder sustainable production of drinking water using the river Meuse as a source. However, the first of the 37 substances is a group of 20 per- and polyfluoroalkyl substances which are currently mentioned in the EU Drinking Water Directive. In addition to the Meuse River Dossier and its Program of Measures that were initiated in the Netherlands to better protect this source for drinking water under the Water Framework Directive, the Clean Meuse Water Chain was started. It is a cooperative venture of Dutch drinking water companies and water authorities as well as the Ministry of Infrastructure and Water Management. Since 2015 the initiative has been working on reducing pharmaceutical residues and industrial substances ending up in the river Meuse.

3.22.P PMT/vPvM Substances: Advances in Identification, Prioritisation, Risk Assessment and Regulation

3.22.P-We291 The impact of Standards in Mobility testing - a case of fragrance materials

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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 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 a 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 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 were selected and evaluated first in the OECD 121 method followed by the OECD 106. During the OECD 121 evaluation it was noted that the standards selected for the screening could potentially have an impact on the results – and this was further investigated and the results are presented in this poster

3.22.P-We292 Applying In silico Models to Screen Persistent Mobile and Toxic Substances in Waste Water Treatment Plants Worldwide

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The new update to the European CLP regulation (EU 2023/707) introduces the PMT and vPvM hazard classes, namely persistent, mobile, and toxic substances and very persistent and very mobile substances, requiring proper management to protect the environment and drinking water. Among emerging contaminants, pharmaceutical and personal care products (PPCPs) may show persistence, mobility, and toxicity, as has been highlighted by several studies. To support PMT identification, regulatory frameworks encourage the use of in silico approaches, such as QSAR models, which can predict multiple properties and activities of chemicals. This presentation summarizes a recent study to investigate PPCPs detected in waste water treatment plants (WWTP)

effluents worldwide, and to assess their PMT properties using various in silico tools. The study reviewed literature from 2014 to 2019, which led to the collection of removal efficiency (RE) data, in different WWTPs worldwide, for 251 PPCPs, and to the classification of these chemicals, according to their RE. PMT properties were assessed using 15 QSAR models. Only reliable predictions were used to identify PMTs according to regulatory criteria. Finally, predicted toxicities and experimental data were combined to calculate the potential environmental risk, for each chemical, following the approach suggested in the OECD-TGD. More than 200 PPCPs were analyzed in more than 70 WWTPs. The application of QSAR models identified 11 PMTs, including antibiotics, psychiatric drugs/antidepressants and a herbicide, which were recognized also among the most persistent classes in WWTPs on the basis of RE data. Limitations in the application of QSARs in this context include the lack of models for the prediction of long-term toxicity, Log_PD, and Log_PK_{oc}, as well as unclear applicability domains. The analysis of potential risk in the aquatic environment led to the identification of 77 individual chemicals posing potential risk in at least one of the different locations included in the study. These results highlight how integrating different in silico models with experimental data can support the identification of PMT chemicals at the screening level. They also highlight critical areas of the chemical domain where substances may exhibit borderline behaviour and QSAR approaches can still be improved to provide more reliable predictions.

Disclaimer/Disclosure:

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3.22.P-We293 Behaviour of Persistent and Mobile Chemicals (PMs) in Aquifer Sediment Microcosms under Different Redox Conditions

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Persistent and mobile compounds (PMs) pose an increasing challenge to the long-term quality of groundwater and the safety of drinking water supplies. Chemical persistence is a key factor in understanding their environmental fate, potential exposure, and associated risks to the environment and human health. However, the persistence and degradability of PMs in groundwater remain poorly understood. Commonly used OECD 308 and 309 simulation tests are primarily applied to surface water and associated sediments, hence most available persistence data are confined to surface water conditions. Compared to surface water, groundwater typically has limited oxygen, reduced microbial activity, and restricted light exposure, which may lead chemicals to behave even more persistently. To address this knowledge gap, we established a 120-day degradation simulation test in aquifer sediment microcosms to evaluate the persistence and biodegradability of 24 groundwater-relevant PMs under three different redox conditions. Additionally, we compared our experimental persistence results with predictions from five commonly used models, as well as available data from surface waters where applicable. 1 mL supernatant was taken from 10 time points, and PMs were quantified using supercritical fluid chromatography coupled with triple quadrupole mass spectrometry. In our study, 22 PMs exhibited half-lives exceeding 40 or 60 days under at least one redox condition, which meets the REACH criterion for chemical persistence in freshwater/marine environments, whereas 12 PMs were persistent under all three tested redox conditions over 120 days. Certain sulfonic acids (i.e., methanesulfonic acid, p-toluenesulfonic acid, and benzothiazole-2-sulfonic acid) were biotransformed only at aerobic conditions and nitrate availability enhanced the biotransformation of two 2-pyrrolidones in anoxic groundwater environment, highlighting the critical role of redox state in persistence assessment. Besides, the five commonly used predictive models exhibited variable performance and low accuracy, likely due to redox-driven or compound-specific degradation behaviour, or the limited availability of relevant experimental data. Our findings emphasise that groundwater compartments with different aquifer properties and prevailing redox regimes should be considered for PMs' regulatory decision-making and in improving modelling prediction.

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3.22.P-We294 Overcoming Chromatographic and Extraction Challenges for the Determination of PMT/vPvM Substances in Water

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The occurrence of persistent, mobile, and toxic (PMT) and very persistent and very mobile (vPvM) substances in the aquatic environment has gained growing attention within the scientific community due to their potential impact on environmental and human health. Their high polarity and strong ionisation mean that many of these substances are poorly retained in most natural attenuation processes. At the same time, routine monitoring programmes still rely largely on analytical methods that are not well suited to the most mobile compounds, and show limited retention for the “gap compounds”. There is therefore a clear need to develop and apply dedicated analytical approaches that can reliably capture these highly polar PMT and vPvM substances in real environmental samples.

This study addresses this challenge by developing and optimising a robust ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for the identification and quantification of representative PMT and vPvM substances in water, and by applying it to groundwater monitoring in Catalonia (north-eastern Spain). A set of 55 substances was selected to represent a broad range of physicochemical and ionic properties. Compounds with logDow < 0 and no robust methods available were deliberately included to challenge method performance. Different chromatographic columns were evaluated and optimised exploring the effects of pH, buffer concentration and aqueous content. In parallel, three water preconcentration strategies were assessed: mixed-mode solid-phase extraction, hydrophilic–lipophilic balanced solid-phase extraction and lyophilisation.

The optimised workflow, with a mixed-mode chromatography, significantly improved retention and peak shape for the most mobile analytes and enabled the determination of previously problematic PMT substances. Application to groundwater samples from the Baix Fluvià alluvial aquifer and the Llobregat River Basin aquifer confirmed the presence of several PMT substances in different hydrogeological sectors, highlighting their relevance in regional water resources. Overall, the study demonstrates that dedicated chromatographic and extraction strategies are essential to close current analytical gaps in groundwater monitoring and to provide more reliable occurrence data for the identification and prioritisation of PMT and vPvM substances in environmental protection and water-management frameworks.

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3.22.P-We295 Persistent and Mobile Chemicals in Groundwater: An Overlooked Challenge for Drinking Water Quality

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Persistent and mobile (PM) chemicals are of particular relevance for groundwater used for drinking water production, as their mobility and low removability allow them to pass through aquatic systems and treatment steps. In our recent screening of 82 groundwater samples from 25 groundwater bodies in Saxony, Germany, 163 PM chemicals were detected, including 72 chemicals reported for the first time in groundwater. Many of these are widely used industrial compounds that have not previously been included in groundwater monitoring. Considering that around 70% of drinking water in Germany originates from groundwater, their presence raises important questions about treatment effectiveness and exposure. To examine their removals during oxidation treatment, a set of priority PM chemicals (selected based on their PMBT, detection frequency, and concentrations) was subjected to Fenton reaction experiments. Several chemicals, including trifluoroacetic acid (TFA), perfluoropropionic acid (PFPrA), and melamine, showed no degradation, demonstrating extreme resistance even under strong oxidation. Some chemicals formed substantial numbers of transformation products (TPs); for example, dibutylamine produced 38 TPs, many of which were probably identified for the first time. In the case of 4-hydroxy-1-(2-hydroxyethyl)-2,2,6,6-tetramethylpiperidine (HHTMP, one of those first-time reported in groundwater), TPs, which are more persistent than the parent compound, including triacetaminine (also found in groundwater) and several additional newly found TPs, were generated. These results suggest that oxidation-based treatment does not reliably mitigate the risk posed by PM chemicals in drinking water and may

rather introduce additional problematic chemicals. Overall, these findings highlight the need for protecting drinking water resources from contamination by PM chemicals rather than relying on technical barriers to remove them.

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3.22.P-We296 Leveraging mass spectrometry data for predicting chemical persistence and mobility via transformer models

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Untargeted high resolution mass spectrometry (HRMS) is widely used for molecular identification and structural characterization, yet only 1–20% of spectra from environmental samples match HRMS/MS libraries (e.g., NIST), leaving most compounds as “molecular dark matter”. Reducing exposure to hazardous substances requires rapid, high-throughput identification of potentially toxic, persistent, or mobile substances. A prior proof-of-concept work (MS2Tox) showed machine learning can predict acute toxicity of unidentified substances from molecular fingerprints predicted from the MS2 data. Building on this, we leverage data-rich HRMS MS2 data directly for property prediction, reducing error propagation and enabling more robust workflows. Beyond predicting toxicity, early detection of environmentally persistent and mobile substances is critical for proactive risk management.

We aim to develop deep learning models that predict environmental persistence and mobility directly from MS2 data acquired in environmental samples, thereby enhancing untargeted screening for deleterious substances. Specific objectives include model development using curated MS2 data, benchmarking against fingerprint-based models, and validation with experimental MS2 data.

HRMS MS2 data were sourced from MassBank (2025.10) and persistence and mobility endpoints from Arp and Hale (2022), based on degradation half-lives and organic carbon-water partitioning (KOC). Compounds with spectra and endpoints were stratified by ionization mode and split into training, validation, and testing sets. Spectra were encoded as m/z-intensity pairs and processed using a transformer architecture inspired by MS2Prop (Voronov et al., 2024). An MS2-derived fingerprint-based QSPR benchmark was implemented for comparison. Predictions on HRMS datasets (e.g., strategic water monitoring) were cross-validated against empirical measurements and PubChem.

We expect MS2-based models outperform fingerprint-based approaches by reducing structural annotation errors and that compounds predicted as persistent or mobile will correspond to known substances documented in literature or databases. This work introduces a novel strategy for predicting persistence and mobility directly from MS2 spectra, advancing quantitative spectra–activity relationships (QSPAR). The approach enhances untargeted screening of environmentally hazardous compounds and may extend to other physicochemical, environmental and biologically-relevant endpoints.

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- (1) INQUIRE (European Commission, Grant No. 31003709), which focuses on advancing innovative methodologies for chemical risk assessment and predictive modeling.
- (2) Mistra SafeChem (Mistra, Grant No. 31005554), a strategic research program aimed at promoting sustainable chemistry and reducing environmental and health risks associated with chemicals.

3.22.P-We297 Development and validation of a GC–MS/MS method for the determination of EPA priority PAHs and metabolites in marine sediments

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Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants in marine sediments and pose significant environmental and toxicological concerns due to their persistence and potential carcinogenicity. Reliable monitoring of the 16 PAHs prioritized by the US Environmental Protection Agency (EPA), together with relevant metabolites, is essential to clarify contamination sources, transformation processes and ecological risks. Nevertheless, their determination in sediment matrices remains analytically demanding because of concentration levels (high and low simultaneously), matrix complexity and the need for high selectivity.

This study describes the development and optimisation of a sensitive GC–MS/MS method using an Agilent system composed of a 7010B triple quadrupole mass spectrometer equipped with a high-efficiency electron impact (EI) source, coupled to a 7890B gas chromatograph fitted with a programmable temperature vaporization (PTV) inlet. The method targets 17 PAHs, including the 16 US EPA priority compounds, together with 20 associated metabolites, such as methylated, dimethylated and trimethylated derivatives, allowing a more comprehensive assessment of degradation pathways, source apportionment and environmental weathering processes. A commercial mixture of six deuterated PAHs was employed as surrogate standards to assess extraction efficiency and method recovery, while a separate mixture of six deuterated compounds was used as injection standards for instrumental variability control.

Chromatographic separation was achieved on an HP-5 column using helium as carrier gas at 1.2 mL min⁻¹. A 1 µL injection volume was applied in splitless mode, with a purge flow to split vent of 50 mL min⁻¹ at 0.75 min. The oven temperature programme was optimized from 100 °C to 300 °C to ensure effective resolution of structurally similar analytes. Acquisition was performed in multiple reaction monitoring (MRM) mode, divided into five-time segments, to enhance sensitivity and discrimination under complex matrix conditions.

The method will be applied to the analysis of marine sediment samples from different locations, providing robust data on spatial distribution and contamination profiles. Particular emphasis was placed on overcoming challenges related to sensitivity, co-elution and matrix effects, demonstrating the suitability of the proposed approach for high-quality monitoring of PAHs and their metabolites in marine sediments.

3.22.P-We298 Monitoring Data as a Tool for Chemical Mobility Assessment: Opportunities, Challenges and Ways Forward

Chesney Swansborough, Claire Gibson and Connor Powell, Vitis Regulatory, United Kingdom

Understanding and assessing the mobility of chemicals in the environment is a critical component of risk assessment, particularly for substances that may pose risks to drinking water sources. Recent developments under EU chemicals legislation, including the inclusion of PMT (Persistent, Mobile, and Toxic) and vPvM (very Persistent, very Mobile) criteria within the CLP Regulation and emerging considerations under REACH, have intensified the need for robust evidence to evaluate the behaviour and impact of mobile substances in the environment and inform regulatory decision-making.

Monitoring data provide essential, real-world evidence of a chemical's presence, transport and persistence in the environment, complementing standard laboratory testing and modelling approaches. Such data can reveal patterns of contamination, identify vulnerable catchments, and highlight exposure pathways that may not be captured through conventional assessments. Despite these advantages, the use of monitoring data in regulatory risk assessment remains limited. Challenges include questions around data quality, representativeness and how monitoring data can be meaningfully integrated into weight-of-evidence approaches.

This poster presentation explores how monitoring data can support, inform and complement the risk assessment of mobile chemicals. This includes:

- Identify key scientific and regulatory barriers to the use of monitoring data, including data quality issues and regulatory acceptance.
- Discuss opportunities to address these barriers through improved data reporting and transparent interpretation and documentation practices.
- Present case studies illustrating how monitoring data may inform assessment of mobile substances.
- Propose a conceptual framework for integrating monitoring data into regulatory decision-making processes and weight-of-evidence approaches.

By stimulating discussion within the SETAC community, this poster aims to contribute to evolving discourse on how monitoring data can be leveraged to strengthen evidence-based mobility assessment and support future regulatory needs under EU chemicals legislation.

3.22.P-We299 Developing a Basin-Scale Environmental Exposure Model for Persistent, Mobile, and Toxic (PMT) substances with Melamine as a Validation Case

Jianguo Liu, Shaoxuan Zhang, Rongjing Lu, Zhichun Zhang and Jiazhe Chen, State Key Laboratory of

The widespread distribution of Persistent, Mobile, and Toxic (PMT) substances is posing increasing threats to human health and aquatic ecosystems. River basins are a major environmental distribution space for PMT substances. Conventional fugacity-based models struggle to accurately describe the environmental behaviour of PMT substances due to their high ionizability and hydrophilicity. Existing activity-based models still use a traditional grid-cell simulating approach that does not adequately match the transport and distribution dynamics of PMT substances in the environment. This study developed a nationwide exposure assessment model for PMT substances based on chemical activity and basin-scale simulation. By establishing basin-scale emission scenarios of PMT substances with 7,388 key parameters related to the industry, population, wastewater treatment and reclamation, and environmental exposure scenarios with over 6,240 key parameters related to the meteorology, hydrology, and multimedia environment in totally 80 secondary basins across China, the model could conduct a national, regional or basin-wide emission and exposure simulation for PMT substances. The model was applied to melamine, a typical PMT substance with a high detection rate in water systems, and validated through nationwide melamine monitoring supplemented by existing monitoring data from 2010 to 2024. The model revealed a pronounced spatial distribution pattern of the emissions and the environmental exposure of melamine across China, and increasing trends in exposure levels across the multimedia environment over the past 26 years. The environmental exposure levels of melamine in regions are strongly related to the activities of major melamine emission industries, such as papermaking and textiles. Areas with higher melamine levels in the environment are clearly reflected through model simulations, i.e., the coastal areas of the Shandong Peninsula, the Taihu Lake Basin, and the Haihe River Basin in the Beijing-Tianjin-Hebei region, suggesting that local governments and the community should give due attention to these findings.

3.22.P-We300 PMT substances in the Human Food Chain: Methodologies for Assessing their Occurrence and Fate

Stefan van Leeuwen, Bas van Dijk, Serena Rizzo, Ruben Meine Kause, Karsten Beekmann, Lianne bouwman, Leonieke van den Bulk and Katja CW van Dongen, Wageningen Food Safety Research (WFSR), Netherlands

Persistent, Mobile, and Toxic substances (PMTs) pose concerns for both the environment and food production. Increasing evidence shows that this class of compounds is present in various water sources. Because water is essential for food production, it is important to understand the occurrence and fate of PMTs in food production systems. Using PMT-contaminated water, such as for irrigation, may ultimately result in PMTs being present in food products. However, many knowledge gaps remain before a conclusion can be drawn regarding these risks.

In this work, we take initial steps to fill such knowledge gaps by (i) taking published PMT prioritisation lists, (ii) establishing analytical chemical analysis approaches based on suspect screening, (iii) studying PMT occurrence in surface water, and (iv) exploring the uptake of PMTs in food crops. Here, we report on steps (i) to (iii). A database containing prioritisation scores for around 10,000 compounds (approach by Hartmann et al. (2023)) was made available by the Dutch National Institute for Public Health and the Environment (RIVM). The scores were calculated based on QSAR predicted scores for P, M, and T properties. Compounds with a PMT-score ≥ 0.33 were considered for chemical analysis.

To prepare for chemical analysis (i.e. suspect screening), relevant information was collected from databases such as CompTox, AMOS (Analytical Methods & Open Spectra), and the Norman SusDat database. Information on liquid chromatography (LC) amenability and electrospray ionisation (ESI) properties was collected, including predicted ions (e.g., $[M+H]^+$, $[M-H]^-$) when experimental data were unavailable. The resulting suspect list, comprising approximately 2,100 compounds, was used both as an inclusion list of masses prioritised for fragmentation during instrumental acquisition on a Thermo Scientific Orbitrap IQ-X Tribrid mass spectrometer and, after acquisition, as a mass list for data analysis to screen for PMTs in Dutch surface water samples.

To avoid selectivity bias, the water samples were only filtered, without further clean-up. Nearly 100 substances were detected, and several of these could be confirmed at confidence level 1 (Schymanski et al. 2014). The results were compared with available occurrence data for Dutch surface waters, such as those reported by RIWA Rijn and RIWA Maas. Future work will study if, and how much PMTs are taken up by food crops. Such data will support risk assessment of PMTs in food production.

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3.22.P-We301 Making the EU's Common Data Platform's database of alternatives to substances of concern an effective tool for substitution of PMT/vPvM substances

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Providing information on existing alternatives to hazardous substances is a key action to support companies in transitioning to safer alternatives. Although alternative databases developed by researchers and NGOs exist, such as Chemsec's Marketplace, the ZeroPM Database of alternatives to PMT/vPvM substances, or the SUBSPORTplus portal, there is currently no EU database aggregating existing information on alternatives to hazardous substances. The current provisional agreement in the EU to establish a common data platform on chemicals however provided that the future platform to be set up and maintained by ECHA will contain 'data on alternatives to substances of concern' and on alternative technologies or materials not requiring such substances (<https://data.consilium.europa.eu/doc/document/ST-10883-2025-ADD-1/en/pdf>).

Data on alternatives to substances of concern in the common data platform could serve two purposes: supporting the work of ECHA's Socio-economic assessment committee (SEAC) in the assessment of the socio-economic impacts of restrictions and applications for authorisation; and supporting companies in finding relevant alternatives for PMT/vPvM substances. To make the platform an effective tool for substitution, it should provide the right information, be designed in a user-friendly way, and contain effective search functions to allow identifying relevant alternatives. It should also allow for scientists and other experts to upload data, so that the platform can be democratized. Herein we present our recommendations for this development in the EU's upcoming common data platform on chemicals.

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3.22.P-We302 Application of a screening level multimedia activity model for evaluating the fate of persistent mobile organic chemicals

Todd Gouin, TG Environmental Research, United Kingdom

In recent years there has been increasing interest to screen and prioritize chemicals used in commerce with respect to their potential to contaminate drinking water sources. The German Environment Agency (Umweltbundesamt – UBA), for instance, has proposed screening criteria to identify chemicals that may pose hazards to drinking water. The hazard criteria aim to prioritize chemicals that are persistent, mobile and toxic (PMT) or very persistent and very mobile (vPvM). The development and application of exposure assessment tools would thus support prioritization activities. This study summarizes the development of a multimedia environmental fate and transport model, which supports the exposure assessment of persistent mobile organic chemicals (PMOCs) under a variety of scenarios, including under steady-state, equilibrium and non-steady-state and non-equilibrium conditions. The developed model includes multilayer soil and aquifer compartments (unsaturated and saturated), which is used to enable a screening level estimate of exposure to PMOCs. Model results, based on an assumption of equilibrium and steady-state, provide an illustrative example regarding the relative importance of including a saturated compartment for screening and prioritizing PMOCs in groundwater that might be used for drinking water, whereas dynamic results provide a relative evaluation of the time needed to exceed toxicological thresholds of concern. Sensitivity and uncertainty analysis demonstrate the importance of system dependent variables, specifically the relationship between the hydrological residence time of an aquifer and the time to exceed threshold values.

3.22.P-We303 Machine learning coupled with causal inference to identify persistent, mobile and toxic pharmaceuticals and disinfectants

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Various pharmaceuticals and disinfectants are utilized in large quantities, with some of these chemicals likely rising concerns on drinking water sources. Persistent, mobile, and toxic (PMT) substances have been recognized as a threat to drinking water resources. Identifying the pharmaceutical and disinfectant compounds of high drinking water concern remains a challenging task due to the lack of high-quality experimental data required for PMT assessment. To solve this problem, we developed and applied a machine learning approach to identify the PMT substances among pharmaceuticals and disinfectants. We constructed and compared different machine learning models by combining molecular description methods, data balancing approaches and machine learning algorithms¹. The optimal model achieved an accuracy of 90.6% based on external test data. Moreover, model interpretation and causal inference indicated that our approach successfully captured the causal relationships between PMT properties and molecular descriptors². Notably, the screening results showed that over 60% of the target pharmaceutical and disinfectant compounds are potential PMT substances, providing a list of prioritized contaminants to prevent unnecessary environmental emission as well as to develop efficient treatment techniques.

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3.22.P-We304 Assessing the State of Science and Developing Best Practices for Mobility Assessment of Difficult-to-Test Substances

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The assessment of substance mobility in the environment has recently become a key regulatory requirement under the European Union's Classification, Labelling and Packaging (CLP) Regulation, following the introduction of new hazard classes for persistent, mobile and toxic (PMT) and very persistent, very mobile (vPvM) substances. These classifications aim to identify chemicals that, due to their persistence and ability to move freely through environmental compartments, may contaminate groundwater and drinking water sources, posing long-term risks to human and ecological health.

Mobility (M/vM) is usually assessed based on the organic carbon-normalized soil–water partition coefficient (KOC). KOC values can be determined through experiments or computer models, but acquiring accurate data is often challenging for many chemicals.

A review has been conducted to assess current methods for mobility assessment, emphasizing the specific challenges presented by two types of substances: charged or ionizable substances and unstable or reactive substances. Charged and ionizable substances undergo complex interactions with soils, including both electrostatic and hydrophobic interactions. Their speciation varies with environmental pH, complicating testing and modeling. Similarly, unstable or reactive substances may transform during testing, making it difficult to determine a meaningful KOC for the original compound and their transformation products.

To address these issues, regulatory experience and scientific literature has been synthesized to offer recommendations for improving the assessment of charged, ionizable, and unstable/reactive substances. Key needs include further investigating the suitability of KOC as a universal indicator of mobility, developing and validating alternative descriptors and test methods for challenging substances, and further guidance for substances that form transformation products. Broader research priorities are also identified, such as improving experimental designs and improving the mechanistic understanding and predictive capability of sorption and transformation processes.

By filling these scientific and methodological gaps, regulators, researchers, and safety assessors can increase confidence in identifying substances with genuine mobility concerns and ensure that the PMT/vPvM framework is applied consistently and defensibly across all relevant chemical classes.

3.22.P-We305 Comparative Evaluation of Koc Determination Methods for Fragrance Ingredients Under New EU Mobility Criteria

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In April 2023, the European Commission introduced a new environmental mobility classification criterion, adopted by the EU Parliament in December 2023, defining substances as Mobile (M) when $\log K_{oc} < 3$ and very Mobile (vM) when $\log K_{oc} < 2$. These thresholds significantly reduce the number of fragrance ingredients classified as M or vM compared to the original proposal ($\log K_{oc} < 4$ for M and < 3 for vM), which would have impacted a higher number of substances.

This project evaluates the applicability of the OECD 121 HPLC screening method for $\log K_{oc}$ determination against a higher-tier approach using freeze-dried sewage sludge as a solid environmental matrix, following OPPTS 835.1110 “Activated Sludge Isotherm” guideline. The objectives are to determine whether sludge-based testing provides more realistic sorption estimates for borderline cases and to assess its applicability for substances with $\log K_{oc}$ values above 3, supporting improved wastewater treatment plant removal predictions in environmental risk assessments.

The experimental setup involves sludge preparation by decantation, centrifugation, freeze-drying, and thermal desiccation. Tests are conducted using varying concentrations of the test substance. Preliminary results indicate that sludge-based isotherm testing often yields higher $\log K_{oc}$ values than HPLC screening, potentially reducing predicted environmental concentrations in surface waters compared to standard *in silico* models.

This work advances K_{oc} determination strategies for fragrance ingredients, enabling more accurate mobility classification and supporting robust environmental risk assessments under evolving EU regulatory frameworks.

3.22.P-We306 The Non-Extractable Residue Problem in Kinetic Analysis

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The assessment of the persistence of an active substance for the purposes of hazard assessment is of increasing significance considering the new hazard classes being introduced via CLP guidance. In particular, the interpretation of bound residue deviates between guidance introduced by FOCUS and the PBT guidance by ECHA, creating a significant disconnect between evaluations, potentially on the same active substance. Here, a number of datasets are evaluated to highlight the impact of applying different handling approaches for NER and discuss the appropriateness of some approaches.

3.22.P-We307 Improving the Determination of the Organic Carbon – Water Adsorption Coefficient (K_{oc}) for Down-the-Drain Chemicals Using a Sewage Sludge Adsorption Assay

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Mobility, under EU CLP legislation, is described as a function of the organic carbon-water adsorption coefficient (expressed as $\log K_{oc}$). Whereas the K_{oc} may be used for non-polar chemicals at a screening level, the K_{oc} remains a simplistic metric and does not consider the full spectrum of complex sorption behaviours that substances can undergo upon contact with environmental matrices. Tier I testing to measure K_{oc} uses the OECD Test Guideline (TG) 121 HPLC method employing a moderately polar stationary phase. The retention time of the test item is compared against that of a range of reference substances, which may or may not be of comparable chemistry. The current Tier II refinement approach OECD TG 106 involves performing adsorption-desorption investigations using a range of soils and/or sediments of different characteristics (at least 5 individual matrices). Although more realistic than Tier I, this approach is time and resource intensive. Furthermore, guidance is required on which K_{oc} value derived from the numerous soils / sediment matrices tested should be adopted as the “representative” K_{oc} value.

Chemicals incorporated within consumer goods, which undergo down-the-drain disposal, will transit via a waste-water treatment plant (WWTP) prior to the effluent entering a waterway. For such products, contact with sewage sludge is the most important adsorption interaction process. Sewage sludge physico-chemical characteristics, including organic-carbon content, tend to be relatively stable in comparison to that of soils and sediments and, therefore, represent a reliable test matrix.

The OPPTS 835.1110 – Activated Sludge Sorption Isotherm TG, therefore, represents a valid Tier II test alternative. It is also representative of the most relevant exposure scenario for down-the-drain chemicals.

Besides establishing a K_{oc} value, this approach also permits calculation of a K_d value (adsorption-desorption distribution coefficient) as well as the direct calculation of a % removal-rate for sewage sludge.

This poster will discuss the relevance and advantages of using the OPPTS 835.1110 TG for the determination of adsorption of down-the-drain chemicals and will compare results derived using the OECD 121 and the OPPTS 835.1110 for a series of fragrance molecules and interpret these results in a legislative and risk assessment context.

3.22.P-We308 OECD TG 309 and the validity criteria for the reference substance: Is there a good season to collect the surface water?

Aminé Tchéré Kakia, Dirk Scheerbaum, Christian Maeß and Kristina Miller, Noack Laboratorien, Germany

For a reliable assessment of the simulation test in surface water (OECD 309), a sufficient microbial activity of the used water should be confirmed. For this purpose, the parallel testing of a reference substance is required and considered for the validity assessment of an OECD 309. The expected time interval for the degradation is usually less than 2 weeks. The challenges around the fulfilment of this validity criterion are well known and most recently subject of discussion.

In this presented work, the mineralization of the reference substance aniline was tested monthly over a period of 14 months (from January 2024 to February 2025) with surface water from the same origin. The purpose was to assess the seasonal difference of the quality (microbial activity) of the surface water and thus its influence on the mineralization rate of aniline.

The aerobic mineralization was performed with radiolabeled aniline over a period of approx. 2 weeks.

The water was collected from the surface of a nature-protected river in Lower Saxony. The tests always started within the sample collection day.

The investigation resulted in different mineralization rates for aniline. The biodegradation data and the associated weather conditions at the time of the sampling are presented and evaluated.

3.22.P-We309 Linking Structure and Hazard: Acute Ecotoxicity of Triazine PMT and PMT-like Compounds

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Persistent, Mobile and Toxic (PMT) substances are increasingly recognised as a threat to water resources. Triazine-based PMT substances are widely used as herbicides and industrial additives, and closely related analogues and degradation products are now detected alongside them in surface and groundwater. It remains unclear whether officially recognised PMT substances are generally more acutely toxic than their structural analogues, or whether structural differences on the triazine ring could predict toxicity.

We addressed this question using acute toxicity tests with zebrafish (*Danio rerio*) embryos and the invertebrate *Daphnia magna*. Thirteen triazine-based compounds were selected around four substances classified as PMT by the German Environment Agency and grouped into three structural classes: methylthio-triazines, chloro-triazines, and melamine-type triazines. In the zebrafish embryo test (ZFET), mortality and malformations were scored at 96 h. *Daphnia magna* immobilization tests were conducted and scored for immobilization at 48 h. Concentration–response models were used to derive median lethal concentrations (LC₅₀; ZFET) and effect concentrations (EC₅₀) for malformations (ZFET) and immobility (*Daphnia*).

The LC₅₀ results support a toxicity ranking among the three structural groups, with methylthio-triazines being the most toxic, chloro-triazines intermediate, and melamine-type triazines the least toxic. These patterns indicate that simple ring features—particularly the position-2 substituent and the presence or absence of N-alkyl side chains—help predict relative acute toxicity among structurally related PMT and PMT-like compounds. Grouping triazine PMT and PMT-like substances by structure can guide early prioritisation but should be complemented by targeted toxicity tests and computational models to distinguish closely related analogues. Together, these insights support structure-informed testing and prioritisation of PMT candidates and can inform the design and selection of safer alternatives.

3.23.P Polychlorinated N-Alkanes – Current State, Challenges and the Way Forward

3.23.P-Th078 Fast Screening of Polychlorinated Alkanes via In-Source Chloride Ion Detection

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Polychlorinated alkanes (PCAs) are high production volume chemicals and globally widespread contaminants, yet their determination remains analytically challenging due to the extreme complexity of technical mixtures. As a result, routine monitoring still lacks fast and broadly applicable screening tools capable of estimating total PCA load without elaborate quantification workflows. This study evaluates whether in-source chloride

formation can provide a faster, more robust, and operationally simple route to quantifying total PCAs in environmental samples.

We developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method that uses in-source fragmentation to generate the diagnostic chloride ions at m/z 35 and m/z 37 from all PCA ions entering the electrospray interface. In-source fragmentation energy and ESI conditions were systematically optimized to maximize the combined chloride-ion signal. The approach was then assessed using commercial single-chain PCA standards (C10-C17; 43-71% Cl) to characterize how chain length and chlorination degree influence fragmentation behavior. As expected, both parameters increased the chloride-ion response, yet all standards provided clear and reproducible signals at low concentrations. Preliminary tests on technical mixtures and spiked environmental samples yielded recoveries of approximately 60–120% relative to high-resolution MS values, demonstrating that the method is suitable for screening-level semi-quantification across diverse matrices.

These findings demonstrate that in-source chloride-ion monitoring allows reliable and efficient estimation of total PCA content, significantly simplifying the analytical workflow. Ongoing work is extending the method to long-chain PCAs, assessing matrix interferences, and further lowering the time of chromatographic separation to support high-throughput environmental monitoring.

Disclaimer/Disclosure:

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3.23.P-Th079 Advanced LC/QTOF-MS Approach for Chlorinated Paraffins: Combining Online SPE and Non-Targeted Screening

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Chlorinated paraffins (CPs), widely used as flame retardants and plasticizers, pose environmental and health concerns due to their persistence and bioaccumulation potential. This study presents a high-resolution LC/QTOF-MS workflow for simultaneous quantitation and screening of short-chain (SCCPs) and medium-chain chlorinated paraffins (MCCPs) in water samples, employing an online solid-phase extraction (SPE) configuration for large-volume injections (500 μ L). The method demonstrated excellent linearity ($R^2 \geq 0.995$) across a seven-point calibration range (0.15–10 $\text{pg}/\mu\text{L}$) and achieved method detection limits below 1 $\text{pg}/\mu\text{L}$ for most congeners, with relative standard deviations $\leq 20\%$. No SCCPs or MCCPs were detected in surveyed river, tap, and treated water samples; however, non-targeted screening revealed surfactants and fatty acids. The workflow enables sensitive CP detection and retrospective analysis, addressing limitations of traditional GC-based approaches for complex CP mixtures. Future work will incorporate isotopically labeled standards and expand to indoor dust and food matrices, supporting comprehensive monitoring strategies for regulated CPs.

3.23.P-Th080 Uncovering the Relative Importance of Chemical Emissions Arising from the Application as Plastic Additives

Chengkang Chen and Frank Wania, Department of Physical and Environmental Sciences, University of Toronto, Canada

Synthetic chemicals can be used simultaneously in various applications including as plastic additives. With the rapid rise in plastic production and the associated pollution, it is vital to investigate the relative contribution of the application as plastic additives to chemical emissions and subsequently to human and wildlife exposure. We select four groups of chemicals, i.e., polychlorinated n-alkanes (PCAs), polychlorinated biphenyls, polybrominated diphenyl ethers, and organophosphate esters, and apply the dynamic substance flow analysis model (CiP-CAFE) to revisit their global historical emissions. We compare emissions of these chemicals arising from their application as additives in plastic products with those from other applications. Using the derived emission estimates as input, we further apply the chemical fate model BETR-Global to assess to what extent emissions arising from plastic additive applications contribute to the environmental exposure to these chemicals globally. Our findings uncover a large contribution of application as plastic additives to total emissions. Taking PCAs as example, 20% of PCA global historical emissions during 1930-2020 are attributed to the use as plastic additives, with this contribution varying between regions and with time. PCA emissions from plastic additives application are more likely to lead to PCA emissions into the atmosphere, while emissions from other

applications are more likely to be to fresh water and soils. Accordingly, plastic additive use of PCA makes a large contribution to global atmospheric contamination. For example, over 60% of short-chain PCAs (SCCPs) in the global atmosphere are attributed to the emissions of SCCPs as plastics additives. This percentage is even higher in China where ~90% of SCCP use is as additive. This work advances our understanding of the vital importance of plastic additives to the emissions of, and therefore ultimately to the human exposure to, many contaminants.

3.23.P-Th081 Tracking Real and Virtual Flows of Polychlorinated n-Alkanes Associated with International Trade Between China and Rest of World

Chengkang Chen and Frank Wania, *Department of Physical and Environmental Sciences, University of Toronto, Canada*

Since the 2000s, China has become the dominant producer, user, and exporter of polychlorinated n-alkanes (PCAs). By 2020, Chinese PCA production accounted for ~40% of global cumulative production. On the one hand, China has exported PCAs and PCA-containing products (e.g., plastics) to the rest of the world, resulting in real flows to, and emissions of PCAs in, other regions (flow 1 (F1)). Also, some PCAs emitted in China are transported to other regions via environmental long-range transport in the atmosphere and marine currents (F2). On the other hand, commodities whose production requires the synthesis and use of PCAs in China are exported to meet consumer demand elsewhere, leading to a virtual emission transfer from consumption regions to China (F3). Finally, some PCA-containing products may be imported into China from consumption regions at the end of their useful lifetime, thus leading to potential emissions during the waste disposal phase (F4). We seek to track and quantify these real and virtual flows of PCAs associated with the international trends of chemicals, products and waste. We apply the dynamic substance flow analysis model (CiP-CAFE) to calculate the global flows and emissions of PCAs for the period from 2000 to 2020 in two scenarios, i.e., with and without considering the trade of PCAs and PCA-related products and wastes between China and other regions. By comparing these two scenarios, we can quantify the impacts of F1, F3, and F4 processes on the emissions in China and other regions. Furthermore, we apply the multimedia fate model BETR-Global to simulate the fate and transport of PCAs, with the derived global emission estimates as input. Again two simulations are performed, i.e., including and excluding Chinese emissions. By comparing these two simulations, we can calculate the magnitude and directions of F2 from China to other regions. The model results shed light on the role of international trade of chemicals and products in transferring globally the emissions of PCAs and therefore the risks associated with exposure to PCAs.

3.24 Recent Advances in Sample Preparation and Related Analytical Methods in Environmental Monitoring

3.24.T-01 Comparing Innovative Sorbents Performances for Passive Sampling of Polar Organic Contaminants through Target and Non-target Screening

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Passive sampling use for monitoring of surface waters presents a valuable alternative for tracking water quality, offering cost-effective time-averaged concentration data, and lowering limits of quantification. Recent studies questioned devices type as criteria of sampling performance, but fewer are considering receiving phase material, apart from the usual HLB sorbent. Targeting polar organic contaminants encompasses a broad range of compounds, including ionized or highly hydrophilic ones. Considering these physicochemical properties is crucial to maximize analytes recovery. Therefore, the development of passive sampling devices filled with cutting edge sorbents mixture appears as a promising approach of passive sampling.

This study investigated the implementation of several combinations of sorbents (ion-exchange and neutral sorbents) in passive sampling devices aimed at polar organic contaminants and particularly the most hydrophilic ones (i.e., drugs, pharmaceuticals and biomarkers). To assess the kinetic parameters of the sorbents mixtures and therefore determine the sampling rates of the tested devices, POCIS were exposed for varying periods (1, 2, 4, 7, 11, 15 and 21 days) in the Seine river, Paris. Grab samples were also collected and extracted through acidic and basic elution steps. Analysis of samples was performed by target HPLC-MS/MS (45 targeted contaminants) and non-target LC-QTOF-MS.

The performance of sorbent combination regarding different target contaminants was assessed. Accumulation of compounds highlights the selectivity of each sorbent combination and enables the estimation of in situ contaminants concentrations. Ion-exchange sorbents performed better with ionized and highly hydrophilic molecules. These are however very selective and exhibit rapid accumulation kinetics, making them less suited to

target a broader range of compounds.

The in-situ calibration of POCIS enabled the determination of sampling rates for a wide range of compounds. The dispersion of values highlights the varying degrees of affinity between compounds and receiving phases. Samples were analysed through non target screening to first compare the detected and identified compounds between the samples within the exposure duration, showing increased detected signals from passive samples with the most extended deployment periods.

Disclaimer/Disclosure:

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3.24.T-02 Innovative Supramolecular Solvent Platforms for Comprehensive Sample Preparation in the Analysis of Chemical Exposomics

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Characterizing the chemical exposome requires analytical workflows capable of handling vast numbers of structurally diverse contaminants spanning wide ranges of polarity, abundance, and matrix complexity. Liquid chromatography-high-resolution mass spectrometry (LC-HRMS) has become the preferred strategy for this purpose. However, the complexity of the analyses and the strong potential for matrix effects, particularly when electrospray ionization is used, make sample preparation the critical step of the analytical process. Conventional organic solvent-based extractions and solid-phase approaches provide partial and biased coverage, depend heavily on analyte polarity, and often require sequential or parallel workflows that compromise throughput and hinder comprehensive chemical elucidation. As a result, large numbers of chemicals are excluded during sample treatment, preventing their detection and ultimately limiting chemical exposome investigation.

Supramolecular solvents are nanostructured liquids formed through the spontaneous self-assembly of amphiphiles. Their intrinsic coexistence of multiple microenvironments, combined with the ability to tune nanostructure, polarity domains, and binding sites, enables the simultaneous solubilization and preconcentration of compounds across wide physicochemical ranges. Evidence from applications in water, indoor dust, urine, and food matrices demonstrates that supramolecular solvents can efficiently extract chemicals across broad polarity ranges, including persistent organic pollutants, pharmaceuticals, flame retardants, bisphenols, and other emerging contaminants. These capabilities directly address the key requirements for exposome-oriented sample preparation: broad chemical coverage, matrix independence, and minimal manipulation.

This work highlights recent advances in the fundamental understanding of supramolecular solvent generation, including the factors governing their synthesis, structural organization, and the modulation of their physicochemical properties. Emphasis is placed on how these insights support the development of extraction processes capable of accommodating contaminants spanning wide polarity and structural ranges. Remaining challenges for widespread routine adoption in analytical laboratories are also discussed, together with the opportunities that supramolecular solvents offer to expand the accessible chemical space and streamline analytical workflows.

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3.24.T-03 Volumetric Dried Blood Spot (DBS) microsampling as smart tool for human biomonitoring of PFAS exposure

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Exposomic is a recent concept defining the integrated compilation of physical, chemical, biological, and psychosocial influences that impact biology. The complexity of measuring the human exposome throughout the lifespan has become evident.

For purpose of human biomonitoring, blood is a particular useful matrix since it allows the detection of biologically relevant circulating xenobiotics. However, sampling of venous blood presents several drawbacks

which can now be overcome by the use of Dried Blood Spot (DBS) microsampling.

This study aimed to develop, optimize, and validate a streamlined analytical workflow based on DBS combined to UHPLC-MS/MS detection of 25 per-fluoro alkyl substances (PFAS), a well-known class of environmental pollutants of concern because of the numerous adverse health effects associated with its acute or chronic exposure.

The sampling approach was based on Capitainer B device, which ensures the collection of a volumetric sample of 10 μL through a microfluidic channel. The extraction is carried out by 30 minutes of sonication after addition of 500 μL of methanol and internal standards. The extract is then centrifuged for 10 minutes, evaporated, and reconstituted by 20 μL of 75:25 aqueous: organic mobile phases. Finally, 3 μL are injected to UHPLC-MS/MS for target analysis. The range of calibration (2.0 – 100 ng/mL) was selected according to clinical guidelines with reference to potential toxicity for human health.

The developed method was validated in terms of specificity, sensitivity (LOD 0.4-1.0 ng/mL), accuracy and precision, fulfilling the acceptability criteria ($\pm 20\%$). Extraction recovery demonstrated excellent values (above 80%), whereas matrix effect reported ion enhancement for 13 molecules ($>50\%$). Lastly, stability testing by ANOVA clearly showed a non-statistically significant difference comparing three levels of temperature (-20°C , 4°C , 25°C) and storage time (1 week, 2 weeks, 1 month).

As proof-of-concept, the method application was performed with a cohort of 10 volunteers residing in Piedmont (Italy). Non-alarming concentrations have been found (ΣPFAS 0.74 – 3.38 ng/mL, mean 1.52 ng/mL).

The minimal invasive volumetric DBS combined with the optimized extraction methodology and a sensitive detection method, reveals to be innovative, rapid, and cheap, and particularly suitable to large biomonitoring campaigns focused on at-risk populations because of occupational exposure or vulnerable-stage of life.

3.24.T-04 Indications towards cooperative degradation of micropollutants in biofilms by mass spectrometric imaging

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There is one major question on biofilm reactor operation: Why are biofilm reactors able to conduct more degradations of pollutants than planktonic/sludge reactors. To answer this question, we degraded benzalkonium compounds in moving bed biofilm reactors (MBBRs). The kinetics of the degradation and the metabolic pathways were elucidated by means of HPLC-HRMS from the bulk water.

The than-known metabolites were targeted for by mass spectrometric imaging (MSI) at the surface of the biofilms with a spatial resolution of 50 μm . Even though, the distribution of the parent compounds was not very homogenous, probably due to different properties of the biofilm surface, be it in charge or in lipophilicity, there were clear maxima of metabolite formation:

A) Several reactions can be localized. It can thus be concluded that the few microorganisms that can metabolize these pollutants are not evenly distributed over the biofilm.

B) The different biotransformations have different reaction centers, especially i) the oxidation of the terminal C by ω -oxidation, followed by both α - and β -oxidation, and ii) the second hydroxylation to form bis-hydroxy derivatives are located differently. Thus, these two different reactions are conducted by different microbial niches. These niches further differed in their lipid patterns, which were measured in the same MSI analysis.

As the different steps of the metabolic chain are located differently, it can be concluded, that different organisms cooperate in this process and probably share the invests into degradation enzymes and possibly the utilisation in energy and carbon harvesting.

Disclaimer/Disclosure:

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3.24.P Recent Advances in Sample Preparation and Related Analytical Methods in Environmental Monitoring

3.24.P-Th082 Intensive Sampling With Ceramic Passive Samplers to Measure Contaminants of Emerging Concern in the Besòs River Delta Groundwater in Barcelona, Spain

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Groundwater is an increasingly important source of drinking water in Europe, but contaminants of emerging concern (CECs) including pharmaceuticals, personal care products and industrial chemicals are only recently being detected, despite their potential harm for the environment. Traditional grab sampling provides only a snapshot of contamination, often missing temporal fluctuations caused by variable river flows, industrial discharges or precipitation events. Passive sampling offers an alternative by integrating contaminant uptake over days to weeks, providing more representative, time-weighted concentrations. Ceramic passive samplers (CPSs) are particularly promising due to their simplicity, robustness, low cost and high sorptive capacity.

In this study, CPSs were successfully calibrated for 54 of 63 target CECs using controlled spiked water experiments, establishing compound-specific sampling rates (R_s) and identifying chemicals with insufficient uptake, such as highly polar compounds or those prone to degradation. Calibrated CPSs were deployed in duplicate in 12 wells and two river sites in and around the Besòs River in Barcelona for 14 days. Grab samples were collected simultaneously at all sites, with two wells sampled intensively seven times during the deployment period to enable direct comparison of time-averaged concentrations.

A wide range of contaminants were successfully detected in the extracts of CPSs, including compounds such as acetaminophen and mecoprop that were undetectable by grab sampling. For other substances, such as 2,4-dichlorophenol and hexamethoxymethylamine, CPSs achieved lower detection limits, enabling measurement where grab sampling failed. Comparison of averaged grab-sample and CPS concentrations in the intensively-sampled wells showed strong agreement: over 80% of compounds matched within the same order of magnitude, with several detected exclusively by CPSs.

These results demonstrate that CPSs can provide a sensitive, reliable and practical tool for monitoring CECs in groundwater, complementing traditional sampling methods. Their ability to capture time-integrated concentrations enhances our understanding of contaminant occurrence and variability in aquifers, supporting improved water quality assessment and management strategies.

3.24.P-Th083 Hydrophobic deep eutectic solvent-liquid phase microextraction as a green method for analyzing environmental contaminants in urine

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Urine is widely used for biomonitoring due to its easy collection, but only a limited range of compounds, due to their low levels, can be reliably measured without extensive preparation. Hydrophobic deep eutectic solvents-Liquid-phase microextraction (HDES-LPME) provides greener and more versatile extraction. This study evaluated menthol:octanoic acid 2:1 (M:C8 2:1), menthol:decanoic acid 2:1 (M:C10 2:1), thymol:octanoic acid 2:1 (T:C8 2:1), and thymol:decanoic acid 2:1 (T:C10 2:1) extraction efficiency of 83 environmental contaminants from human urine. Among them, menthol:octanoic acid 2:1 showed the highest extraction efficiency and was selected for further optimization and validation at four concentration levels (0.5, 1.0, 5.0, 10.0 $\mu\text{g L}^{-1}$), followed by LC-MS/MS quantification through HPLC-MS/MS 1290 Infinity II HPLC system coupled to an Agilent 6475 Triple Quadrupole Mass Spectrometer. HDESs were prepared by stirring the components at 60°C to obtain eutectic liquids. Firstly, 1 mL of urine was pretreated with β -glucuronidase. For the LPME, 300 μL of HDES was added to urine, sonicated for 10 min and 5 min centrifuged at 10,000 rpm. Thus, 100 μL of 1.5 M NaOH was added dropwise to the HDES layer, and the mixture was manually shaken. Then 100 μL of 0.5 M citric acid was added. The mixture was again centrifuged for 10 min at 10,000 rpm to separate the phases. Finally, 150 μL from the HDES phase was diluted 5-fold with methanol before the injection. M:C8 2:1 showed average recovery ($80 \pm 22\%$) and extraction ability (58 out of 83 analytes with recoveries above 75%), higher than M:C10 2:1 and the thymol-based HDESs, and was chosen as the most effective extraction solvent. The trueness of the procedure was estimated by the recoveries for a contaminant-free urine, spiked with four concentration levels of analytes. For all concentration levels, a large part of the recoveries span a range of 60 to 120%. A certain variability was observed for organophosphates (30-118%) and pyrethroids (17-101%). Recovery rates for Chlorophenoxypropionic acids and neonicotinoids were lower than 60%. The method was applied to 22 adolescent and 8 adult urine samples from the Barcelona metropolitan area. Bisphenols, benzophenones, and parabens were detected at 0.15–9.15, 0.01–2.52, and 0.02–9.10 $\mu\text{g L}^{-1}$, respectively. Overall, the HDES-LPME procedure using M:C8 2:1 proved effective for broad-spectrum contaminant extraction from urine and was suitable for real biomonitoring applications.

3.24.P-Th084 An Optimized Spectrophotometric Approach Based on p-Dimethylaminobenzaldehyde for Reliable Urea Monitoring in Environmental and Industrial Samples

Reliable quantification of nitrogenous compounds such as urea is critical for both environmental assessment and industrial process control. In this study, an optimized spectrophotometric method was developed using p-dimethylaminobenzaldehyde (PDAB) as a derivatizing reagent under controlled acidic conditions. The approach was designed to overcome reproducibility issues related to the aging of acidic PDAB solutions, variations in analytical parameters, and the detection of low urea concentrations. The color reagent was prepared by dissolving PDAB in a 1:1 (v/v) mixture of glacial acetic acid and water, followed by the addition of concentrated sulfuric acid (H₂SO₄). A Central Composite Design (CCD) within the framework of Response Surface Methodology (RSM) was employed to optimize the reagent composition, identifying an optimal PDAB:H₂SO₄ molar ratio of 1:0.89, which produced the maximum absorbance at 430 nm for a 10 mg L⁻¹ urea concentration. Kinetic evaluation confirmed the stability of the chromogen formed at low urea levels, supporting high-throughput laboratory operation. The method was validated according to the Standard Methods for the Examination of Water and Wastewater (Part 1040), exhibiting excellent linearity ($R^2 > 0.99$), precision indicated by inter-laboratory relative standard deviations below 5 %, and recovery between 90 % and 110 % at three concentrations (10, 50, and 100 mg L⁻¹). The limits of detection and quantitation were 2.2 mg L⁻¹ and 10 mg L⁻¹, respectively. The procedure demonstrated strong robustness with minimal sensitivity to experimental variations and provided an efficient means to minimize interferences from ammonia and hydrazine compounds commonly present in fertilizer effluents. Overall, this enhanced PDAB-based approach provides a simple, sensitive, and reproducible analytical tool for the monitoring of urea in diverse matrices. Its adaptability for both environmental samples and fertilizer industry applications makes it a practical and sustainable alternative to more time-consuming conventional techniques.

Disclaimer/Disclosure:

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3.24.P-Th085 Determination and Occurrence of Ethylene Oxide and 2-Chloroethanol in Foods

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Ethylene Oxide (EtO) is a gaseous compound permitted as a fumigant for certain crops in several countries, including the United States, Canada, and India. Owing to its carcinogenic and mutagenic effects, the European Union (EU) has banned its use as a pesticide since 1991, and Taiwan likewise prohibits its residues in foods. Since 2020, the EU has reported numerous incidents of EtO contamination, mainly involving nuts, soybeans, and wheat products, which are important dietary staples and major imported food items in Taiwan. EtO readily reacts with chloride ions and water in food matrices to form 2-Chloroethanol (2-CE). Accordingly, the EU defines the residue level as the sum of EtO and 2-CE, expressed as EtO equivalents. Building on the currently adopted regulatory approach, this study refined and extended the analytical methods to nuts, soybeans, and wheat products, and investigated their EtO and 2-CE concentrations using a representative sampling design based on the core food approach. Matrix-matched calibration curves for each food type showed linearity, recovery, and relative standard deviation values that complied with regulatory validation criteria. The method detection limits for 2-CE ranged from 0.002 to 0.01 mg/kg, corresponding to 0.001–0.0055 mg/kg as EtO equivalents. Concentrations in sesame, nuts, dried tofu, soy milk, cookies, and noodles were all within regulatory limits. This study combined a refined analytical method with a representative sampling design to enhance accuracy and reliability of EtO and 2-CE residue monitoring in foods.

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3.24.P-Th086 Revealing Hidden Thiol-Containing Molecules in Sediments: A Novel Extraction Strategy Based on Thiol–Disulphide Exchange and Its Application to OECD 219 Tests

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Environmental pollutants such as pharmaceuticals, personal care products, and pesticides are a growing concern worldwide due to their potential effects on ecosystems and human health. Assessing their behaviour in soil and sediment environments requires a combination of chemical and biological analyses, which can be time-consuming and resource-intensive. Quick, comprehensive, and cost-effective methods are therefore needed to improve environmental monitoring and risk assessment—especially for compounds that can strongly interact with sediment matrices.

Among these, thiol-containing molecules represent a particular challenge. Due to their high reactivity, they can form disulphide bonds with naturally occurring thiols in soil and sediment, leading to incomplete extraction and underestimation of their environmental concentrations. To address this issue, we developed a novel extraction method based on the thiol–disulphide exchange reaction, enabling recovery of both free and bound thiol species. As a case study, the extraction method was optimized and validated for Thiorphan, the active thiol metabolite of the pharmaceutical Racecadotril, within the framework of the Sediment-Water Chironomid Toxicity Using Spiked Water (OECD 219). Using β -mercaptoethanol as a reducing agent, the approach efficiently releases sediment bound Thiorphan, which is then extracted and quantified via HPLC–TQ in MRM mode. The method exhibited excellent analytical performance, with a linear range of 10–1000 $\mu\text{g/L}$, a detection limit of 10 $\mu\text{g/L}$, 95% accuracy, and 2.6% precision (RSD).

This approach provides a new analytical tool for detecting reactive thiol-containing pollutants in soil and sediment. It is particularly relevant for ecotoxicity studies in soil and sediments (e.g. OECD 219 test), in which it will support a better understanding of pollutant fate, persistence, and bioavailability.

3.24.P-Th087 Fast und automated Microplastics Analysis in Carbonated Beverages by QCI based LDIR- A critical Evaluation of Spectral Range Selection and Size Detection Limits

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Microplastics have become an unwelcome ingredient in food and drink, raising concerns about how plastic materials contribute to contamination. This study demonstrates a rapid, automated approach for detecting and characterizing microplastics in carbonated beverages and apple juice using an Agilent 8700 Laser Direct Infrared (LDIR) chemical imaging system. Samples packaged in various materials such as plastic, glass, aluminum cans, and multilayer cartons were analyzed directly on aluminum-coated filters, a process requiring minimal sample preparation. Using the Particle Analysis method and inbuilt microplastics spectral library within the Agilent Clarity software, microplastics were detected in all beverage types. Polyvinyl chloride (PVC) and polyethylene terephthalate (PET) were identified as the most prevalent polymers. The workflow, including robust quality control and direct-on-filter analysis, highlights the suitability of the 8700 LDIR for microplastics monitoring in food and beverage applications.

Furthermore, critical aspects such as the selection of the spectral range and the influence of interferences such as natural PA and Mg stearate will be examined and critically discussed in this presentation. The LDIR 8700 was compared with other systems such as the Cary 620 FPA Imaging System and the Cary 630 FTIR spectrometer, and the spectral range was compared to the fingerprint range for all systems. In all cases, polymers were identified with high HQI. The presentation will also demonstrate strategies to identify PA in presence of PA naturally occurring and Mg Stearate in presence of PE.

3.24.P-Th088 Development of Green, Comprehensive Analytical Methods for Monitoring Organic and Inorganic Disinfection By-Products in Drinking Water

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Disinfection of drinking water, while essential for public health, results in the formation of a complex mixture of regulated and emerging disinfection by-products (DBPs). The new European Union (EU) regulation (EU 2020/2184) requires improved analytical capacity for DBP surveillance. This work addresses the challenge of developing sensitive, high-throughput, and sustainable analytical strategies for the determination of diverse DBP classes: volatile organic trihalomethanes (THMs), polar organic haloacetic acids (HAAs), and inorganic anions (IAs).

To analyze THMs, three advanced green microextraction techniques were investigated: high-capacity sorptive extraction (HiSorb), thin film-solid phase microextraction (TF-SPME), and stir bar sorptive extraction (SBSE). Among these, the headspace (HS)-HiSorb method coupled with Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC-MS) was optimized. Although the combined use of TF-SPME and SBSE yielded a

synergistic effect, achieving the highest extraction efficiency for THMs, the optimized HiSorb method-using a polydimethylsiloxane (PDMS) sorbent- offered the best balance between high performance and practical suitability for routine, automated monitoring.

For the analysis of nine HAAs and the three inorganic anions (chlorite, chlorate, and bromate), two separate Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) methods were successfully developed. This approach eliminated the traditionally required time-consuming derivatization or extensive sample pretreatment steps for GC-MS analysis of HAAs, simplifying the workflow significantly. Both LC-MS/MS methods achieved satisfactory low limits of quantitation (LOQ) (e.g., bromate: 5 ppb; HAAs: 5-50 ppb) and demonstrated high linearity ($R^2 > 0.99$), ensuring compliance with EU regulatory limits.

This integrated analytical strategy provides a robust and validated toolbox for the concurrent quantification of a wide range of organic and inorganic DBPs. The demonstrated utility of combined green microextraction with direct LC-MS/MS analysis represents a significant advancement toward sustainable, high-throughput environmental monitoring in water quality assessment.

Disclaimer/Disclosure:

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3.24.P-Th089 Novel Untargeted Two-Dimensional Mass Spectrometry Based Environmental Analysis

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Environmental pollution is hazardous to human health with wide ranging impacts on the biosphere in general. One of the key challenges of understanding environmental pollution is the complexity of the sample. These samples are composed of thousands of compounds, which interact with each other to further increase the complexity of the sample as it ages. The majority of components are routinely missed by targeted analyses, which results in poorer understanding of environmental pollution.

To overcome these limitations, untargeted analysis combining chromatography and high-resolution spectrometry are commonly employed. However, it can be time-consuming to develop suitable chromatographic methods, requiring experienced scientists, and the cost of acquiring appropriate hardware such as Fourier-transform ion cyclotron resonance mass spectrometers (FT-ICR MS) or orbitrap mass spectrometers is very high. This reduces the accessibility of untargeted analysis to many labs.

Two-dimensional mass spectrometry (2D-MS) is an emerging technique that performs rapid, untargeted analysis of complex unknown samples without the need for chromatography. Instead, 2D-MS employs encoding techniques to correlate precursor compounds with their fragments without prior precursor isolation. Quadrupole two-dimensional mass spectrometry (Q-2DMS) enables 2D-MS on benchtop instruments such as quadrupole time-of-flight (Q-ToF) mass spectrometers, reducing the cost and increasing the accessibility of 2D-MS.

In this work we apply this novel technique to multiple environmental applications with a view to demonstrating a lower-cost approach to untargeted analysis and increase its accessibility. Samples were acquired from different environmental sources using simple, low-cost methods and minimal sample clean-up to maximise the number of components identified in samples. Additionally, work is ongoing to use unique fingerprint patterns in the data-rich 2D-MS plots for source attribution.

3.24.P-Th090 Comprehensive Identification of Emerging Contaminants in Tap Water from 12 Countries Combining Supramolecular Solvent Extraction and Suspect Screening Analysis

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The growing production of chemical compounds has led to the massive release of substances into the environment, including a wide range of organic pollutants detected in wastewater effluents. Many of these compounds lack chemical and toxicological information and are therefore classified as emerging contaminants (ECs). Wastewater effluents are discharged into various water bodies, serving as an entry pathway for ECs into the hydrological cycle and promoting their global dispersion. Since drinking water treatment plants rely on these water bodies and are equipped with treatment processes designed to remove only specific contaminants, the presence of ECs in tap water must be assessed.

High-resolution mass spectrometry (HRMS), due to its high mass accuracy, enables the identification of compounds without the need for analytical standards. However, the success of non-targeted HRMS

methodologies depends on the implementation of extraction techniques capable of covering compounds with high structural diversity and a wide polarity range. Water analysis using conventional solvent-based methods is limited to the extraction of non-polar and medium polar ECs and requires additional purification steps to achieve adequate selectivity.

In this context, the use of supramolecular solvents (SUPRAS) was proposed as a strategy for the multiextraction of ECs in 53 tap water samples collected from 12 countries. The intrinsic properties of SUPRAS combined with suspect screening analysis (SSA) enabled the tentative identification of 33 ECs across a wide polarity range (logP -1.3 to 16.2), belonging to different chemical classes, including cleaning and household care products, industrial chemicals and intermediates, personal care products, plasticizers, stimulants, surfactants, and others. Most of these ECs have not been previously reported in tap water. These results highlight the remarkable extraction capabilities of SUPRAS, offering a broad-spectrum, streamlined, and efficient alternative to conventional protocols. This innovative methodology reinforces the advantages of supramolecular solvents as a versatile tool for identifying overlooked contaminants and exploring new exposure pathways in urban environments.

Disclaimer/Disclosure:

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3.24.P-Th091 DPX-Based Sample Cleanup for the Analysis of Emerging Contaminants in European Eel Brain

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Since the mid-20th century, the European eel population has been reduced to a minimal presence worldwide. It is estimated that in the last 50 years the effects of this extremely vulnerable species have been reduced to less than 95%. The main causes include overfishing, disruption of ecological connectivity, and parasitic infections from bacteria, viruses, and macroparasites. Climate-driven changes linked to industrialization and globalization have further accelerated the decline of Mediterranean eel populations.

Recently, the high chemical contamination of water including the presence of heavy metals, drugs and pesticides seems to be implicated in the decline of European eel stocks in the last decades. Many of these pollutants can alter the normal functioning of the nervous system, induce lesions and behavioral deficits that affect migration and reproduction. However, information on levels of potentially neurotoxic pollutants in the eel brains is still very scarce.

The goal of this work to investigate the bioaccumulation of several neurotoxic pollutants including pharmaceuticals, pesticides, and drugs of abuse in European eel brain samples from the Pyrénées-Orientales. For the extraction approximately 20-200 mg of brain tissue was homogenized with a bead beating machine using 0.5 mL of cold acidified acetonitrile/methanol, followed by a 15-min ultrasonic bath and centrifugation. For purification, the effectiveness of dispersive pipette extraction (DPX) cleanup was evaluated using the HybridSPE DPX Tips. To increase throughput and reduce manual errors, the Hamilton Robotics platform was employed, requiring low volumes of sample and organic solvents, time, and less sample manipulation. The method was applied to samples from the lower part of the Têt y Tech rivers and the Verdoube river, Canet-Saint-Nazaire and Salses-Leucate lagoons, lower part of Llança river, Els Aiguamolls de l'Alt Empordà lagoons, and Aiguamolls del Baix Ter Girona) and Barcelona (lower parts of Tordera and Besòs rivers and Llobregat Delta river ponds).

Identification and quantification of compounds were carried out by liquid chromatography coupled to Thermo Orbitrap Xploris 240 mass spectrometry system, by applying sophisticated wide-scope target screening workflows.

The automated DPX tip cleanup procedure was very fast allowing for simultaneous analysis of many samples in few minutes. Results revealed the presence of several pollutants, especially pesticides, in eel brain tissues.

Disclaimer/Disclosure:

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3.24.P-Th092 Development of a method for quantifying essential and toxic elements in whole blood for biomonitoring studies

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Essential and potentially toxic elements play critical roles in metabolic processes and are closely associated with the prevention or onset of various health disorders. Biomonitoring studies help define safe reference values, which are crucial to guide effective public health policies. However, the analysis of blood requires robust method development to overcome matrix complexity and ensure reliable quantification of these challenging trace elements.

This study aimed to develop a simple dilute-and-shoot Inductively Coupled Plasma – Tandem Mass Spectrometry (ICP-MS/MS) method capable of simultaneously quantifying essential and toxic elements in whole blood with high sensitivity. To enhance method reliability, validation will be conducted following the International Council for Harmonisation (ICH) M10 guideline for bioanalytical method validation.

The methodology involves a mild digestion and dilution step (dilute-and-shoot) using 0.5% (v/v) nitric acid and 0.01% (v/v) Triton X-100. Different instrumental conditions, including collision/reaction cell gases, internal standards, and dilution strategies, were evaluated to improve accuracy in this complex matrix. Method development included assessment of potential matrix effects, evaluation of linearity, limits of detection, instrumental and analytical precision, and accuracy studies using certified reference materials.

The method enabled the quantification of 13 essential and toxic elements with improved detectability and reduced matrix-induced suppression. Polyatomic interferences were effectively minimized through the use of reaction and collision gas modes, producing results suitable for biomonitoring applications.

This straightforward dilute-and-shoot approach demonstrates strong potential for routine multielement analysis in whole blood and can support large-scale biomonitoring efforts, contributing to the establishment of robust reference values and supporting public health decisions related to nutritional status and chemical exposure.

3.24.P-Th093 TIMFIE Sampling Offers Time-Integration and Quantitative Determination of Multiclass Organic Micropollutants in Whole Water

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Organic micropollutants, e.g. pesticides, pharmaceuticals and PFASs pose a risk to aquatic organisms, ecosystems and their functions. Their presence and concentrations in the environment vary over time and sampling techniques that enable determination of average levels in whole water are vital to estimate chronic exposure of biota and assess waterbody status.

Time-Integrating, Micro Flow, In-situ Extraction (TIMFIE) is a novel sampling technique that meets the need for: whole water analysis, quantitative methods possible to validate according to established principles, fully time integrated from continuous active sampling, low cost and flexible use.

TIMFIE samplers are made from inexpensive laboratory consumables and requires no battery or power supply. A 120-mL syringe is set under negative pressure by pulling and locking the piston with a nail. A flow restrictor (narrow bore PEEK tube) enables a low water flow through one or more solid phase extraction (SPE) cartridges, where target compounds are continuously extracted in situ over one or two weeks. The extracted sample volume is accurately determined which is fundamental for subsequent quantitative analysis. Sampling flow rate (and thus possible sampling time) is easily controlled by varying flow restrictor back pressure.

Two polymer columns in series with a weak anion exchange material (HR-P, HLB, HR-XAW, Macherey-Nagel) were chosen to enable broad extracting capability. The SPE assembly was handled as one unit during conditioning and elution steps, and final extract split for subsequent analysis with four LC-MS/MS multimethods for different compound classes (here 119 pesticides, 66 pharmaceuticals, stimulants, industrial chemicals and personal care products, and 30 PFASs). Methods were validated with a 50 mL sample volume and a final extract volume of 0.5 mL resulting in a theoretical concentration factor of 100.

The SPE cartridge format, with Luer connections in both ends, enables automation of conditioning and elution steps using in-house made manifolds and a stand-alone LC-pump. This significantly reduces tedious lab-work and minimizes handling variability and errors.

Due to its compact and robust design, the TIMFIE sampler can be deployed using various methods, with

submerged placement being preferable since it provides more stable environmental conditions and minimizes visibility to passersby.

3.24.P-Th094 Determining Water Solubility of Poorly Soluble Liquids; Beyond OECD 105

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Water solubility of compounds is an essential parameter to understand how a chemical behaves in the environment and any hazards that may be associated with it, while also being a requirement in global chemical assessments and regulatory submissions. The OECD 105 guideline currently uses two methods for determining water solubility: shake-flask and column elution. Both methods have their limitations, with shake-flask being suitable for compounds with a preliminary solubility of >10 mg/L, while column elution is suitable for low solubility compounds (<10 mg/L), but is unsuitable for testing liquids.

Here we present a successful case of determining water solubility using an alternative, slow stir method. This method uses principles taken from the OECD 123 guideline (partition coefficient; slow stir method), along with criteria taken from the OECD 105 guideline, to determine the water solubility of a poorly soluble, liquid compound. A previous study demonstrated the effectiveness of this methodology for determination of water solubility for a medium-chain saturated hydrocarbon compound; in this case, however, it was applied to measure the solubility of a long-chain fatty acid methyl ester. So far, experimental investigations have focused on compounds with densities lower than 1 g/mL, however with appropriate modifications to the experimental design, this method may also be applied to compounds with densities greater than that of water.

For substances with densities of less than 1 g/mL, the compound is directly dosed onto the surface of the water in an appropriate vessel, stirred slowly until a minimal vortex is formed, and sampled at relevant time points until an equilibrium is reached. Analysis and data handling followed the recommendations described in the OECD 105 guideline. The work therefore presents suitable methodology for the determination of poorly soluble liquids.

Disclaimer/Disclosure:

Previous work conducted by: D. Letinski, A. Redman, H. Birch, P. Mayer, Inter-laboratory comparison of water solubility methods applied to difficult-to-test substances, BMC Chemistry, 2021, 15:52, DOI: 10.1186/s13065-021-00778-7

3.24.P-Th095 Evaluating a New Polymer-Based Device for Dried Urine Microsampling of Environmental Organic Contaminants

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Microsampling strategies have become practical alternatives for biological sample collection in remote or logistically constrained settings. Although dried blood formats are well established, dried urine microsampling remains underexplored, particularly for the determination of environmental organic pollutants. The newly developed Ouro Health Polymer, created by Reload Health in collaboration with the Analytical and Systems Toxicology Laboratory (ASTox – USP), offers a novel platform for passive urine adsorption, drying, and field-ready biomonitoring.

This study evaluates the analytical feasibility of the Ouro Health microsampler for detecting multiple classes of environmental pollutants in dried urine, aiming to develop and optimize a polymer-based method for their quantitative determination.

Synthetic urine was spiked with target pollutants and absorbed onto the polymer by immersion during 5 min with gentle agitation. The multilayer structure enables passive drying at room temperature in 12 hours. Extracts were recovered using 7 mL of solvent, followed by SPE cleanup. After derivatization, samples were analyzed by GC-MS to assess recovery and detectability of bisphenols, parabens, and benzophenones. Chromatographic separation was carried out on a DB5-MS column. The oven temperature of the chromatographic column was initially maintained at 70 °C for 2 min, followed by a heating ramp from 15 °C/min to 210 °C, with a subsequent increase of 20 °C/min to 280 °C, holding for 5 min. Finally, a rise of 10 °C/min to 300 °C, was held for 10 min. UPLC-MS/MS will also be tested to expand the evaluation of polymer performance.

We hypothesize that the polymer provides efficient adsorption, stabilization, and extraction of organic pollutants, supporting sensitive GC-MS quantification and improved analytical performance through optimized extraction and instrument conditions.

This work represents the first evaluation of the Ouro Health polymer for environmental biomonitoring, demonstrating its potential applicability not only to dried urine but also to other non-biological liquid samples, such as environmental water. The results highlight its potential for low-infrastructure sampling, particularly in remote areas where conventional urine collection, refrigeration, and transport remain challenging.

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3.24.P-Th096 Automation of SVOCs Analysis in Water Using EPA 8270: A High-Throughput Workflow with GC-MS/MS and Robotic Sample Preparation

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Monitoring semi-volatile organic compounds (SVOCs) in water is critical for environmental compliance and public health. Traditional EPA 8270 workflows rely on manual sample preparation, which is time-consuming, solvent-intensive, and prone to variability. We present a fully automated solution integrating robotic sample preparation with Agilent 8890 GC and 7010C triple quadrupole MS/MS for the analysis of a great number of compounds, including PAHs, PCBs, pesticides and chloronaphthalenes. The method employs two optimized workflows—acidic for most analytes and basic for amines—combined with matrix-matched calibration to ensure regulatory compliance with EPA 8270 performance criteria. Results demonstrate excellent linearity ($R^2 > 0.99$), recovery (70–130%), and precision (RSD $< 10\%$ for most compounds), even at trace levels (5–10 ppt for PAHs and PCBs). Automation significantly reduces solvent use, waste generation, and operator exposure while improving throughput and reproducibility. This robust, high-sensitivity approach enables cost-effective, scalable SVOC monitoring in environmental water matrices.

3.24.P-Th097 Development and optimization of a sample preparation method for the determination of organic micropollutants in soil samples using GC-APCI-QTOF MS

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Growing awareness of organic micropollutants (OPs) in soil necessitates robust analytical methods, as complex matrices and diverse OP mixtures often lead to underestimated concentrations without comprehensive chemical coverage. Wide-scope sample preparation enables simultaneous analysis of emerging and legacy OPs, increasing analytical efficiency while reducing environmental impact. Gas chromatography with atmospheric pressure chemical ionization quadrupole time-of-flight mass spectrometry (GC-APCI-QToF MS) offers high sensitivity and selectivity, while enabling wide-scope screening for monitoring soil quality. The scope of this study was the development of a generic sample preparation protocol aimed at OPs in soil samples, maximizing the capabilities of cutting-edge instrumentation. Thus, three sample preparation methods, Accelerated Solvent Extraction (ASE), Ultrasonic Assisted Extraction (UAE), and modified QuEChERS (mQuEChERS), were tested using a smart validation scheme of 38 compounds selected as representative OPs across major pollutant classes: polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated naphthalenes (PCNs), and organochlorine pesticides (OCPs). The mQuEChERS method emerged as the most suitable, employing solid-liquid extraction assisted by sonication, followed by phase separation and solid phase extraction purification, leading to optimized analyte recovery and matrix interference reduction. The mQuEChERS protocol was validated for 75 analytes spanning emerging and legacy OPs, including 16 PAHs, 19 PCBs, 5 PCNs, 16 OCPs, and 19 pesticides. The method demonstrated excellent linearity over 30–300 $\mu\text{g/kg}$, achieved recoveries of 70–120%, and maintained high precision (RSD below 11%). The final validated method was applied to real-world soil samples collected from Attica, Greece, and analyzed via GC-APCI-QToF MS. In total, 31 OPs were detected at ultra-trace levels, 16 PAHs, 2 PCBs, 5 OCPs, plus 8 pesticides. PAHs displayed the highest concentrations, with phenanthrene, pyrene, and naphthalene being the most prevalent. Pesticides, like Pendimethalin and Amitraz, followed, while OCPs like DDT were present at lower levels. Results reflected the impact of local industrial and agricultural activities on the environmental contaminant profile. The validated

mQuEChERS stands out as a robust, efficient, and environmentally friendly method, expanding monitoring capabilities worldwide when combined with GC-HRMS.

3.24.P-Th098 Passive Sampling of PFAS Across the Water Cycle: Calibration, Matrix Effects, and Source Identification

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In the recent years passive samplers have become a powerful tool to detect per- and polyfluoroalkyl substances (PFAS). In contrast to grab sampling, passive sampling can provide time weighted average concentrations and is particularly suitable for detecting low and fluctuating concentrations of the target analytes. However, there remain uncertainties of applying passive samplers for PFAS in complex water matrices, such as wastewater (WW). This study aims to address the matrix effects, evaluate their influence on the PFAS sorption and calibrate the passive sampler in the field, to enable source identification across diverse aquatic environments. We have conducted laboratory experiments to test the matrix effects of pH, salinity, humic acids and organic matter of the PFAS sorption on a weak anion exchange sorbent. Increased PFAS sorption was observed at moderately elevated pH, salinity, and humic substances within the tested ranges, whereas the lowest sorption occurred at the lower ends of these ranges. Finally, polar organic chemical integrative samplers (POCIS) with our chosen sorbent were deployed long-term deployment in surface and drinking water and short-term deployment in WW. In-situ calibrations were performed under linear uptake conditions to obtain sampling rates for the different water matrices and the time-weighted average concentrations were determined. This combined laboratory and field approach improves our understanding of matrix effects during PFAS monitoring and allows deviations from optimal conditions to be accounted for during in-situ application. Overall, our findings extend the applicability of passive samplers to complex water matrices and thereby contribute to comprehensive PFAS source identification.

Disclaimer/Disclosure:

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3.25 Safe Organic Waste Circularity: Monitoring and Managing Contaminants in Valorisation Processes

3.25.T-01 A Smelly Situation – Assessing Pollutant Profiles of Sweden’s Most Used Organic Waste-Based Fertilizers with an Accelerated Solvent Extraction, GC-HRMS, and CALUX® Bioassay-Based Combination Method

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Reusing organic waste-based fertilizers is promoted to return key nutrients (phosphorus, nitrogen, organic matter) to agricultural soils, yet emerging micropollutants, particularly in sewage sludge, raise concerns. This study compares pollutant profiles of the three most used organic waste fertilizers in Sweden: sewage sludge, agro industrial waste fermentate and cow manure. 12 sludge samples from Swedish wastewater treatment plants (WWTPs) with varying catchment sizes and treatments (n=3) as well as 5 fermentate samples and 5 manure samples were included.

Using a previously developed, comprehensive method, samples were lyophilized, extracted using accelerated solvent extraction with a n-hexane and dichloromethane mix and analyzed by GC HRMS with suspect and non target screening (NTS). DR and Nrf2 CALUX® bioassays measured activation of the aryl hydrocarbon receptor and oxidative stress, expressed as Bio TEQs and Bio CEQs based on 2, 3, 7, 8–TCDD and curcumin standards respectively.

A first assessment with suspect screening at confidence level 1 indicates a higher overall pollution of sewage sludge than agro-industrial fermentate and manure. We screened for 46 compounds including polychlorinated biphenyls (PCBs), dioxins and furans (PCDD/Fs) as well as polycyclic aromatic hydrocarbons (PAHs), phthalates, siloxanes and plastic additives using native batch standards. The phthalate DEHP occurred in all sludges and fermentates, while DIDP appeared only in sludges. Plastic additives were present in sludges and fermentates. Manure showed a single detection (siloxane D5).

DR CALUX® activities expressed as Bio-TEQs were highest in sludge (0.5–15.3ng/g), followed by fermentates

(0.3–1.7ng/g) and lowest in manure (0.3–0.8ng/g). Yet, no PCBs or PCDD/Fs were detected; among nine PAHs screened, anthracene, chrysene, and benzo[a]pyrene occurred in some sludges, underscoring the value of complementary bioanalytical tools. Nrf2 Bio CEQs ranged 152–1216µg/g in sludges and were lower in fermentates (153–856µg/g) and manures (65 and 121µg/g), with 3 manure samples <LOQ. Sludges from city WWTPs showed the strongest responses in both assays.

Overall, sludge, particularly from cities, exhibited the highest DR and Nrf2 activity as well as a higher number of detected pollutants than fermentates and manure. No clear trend in detected pollutants or activity was observed across sludge treatments. Suspect and NTS with in-house libraries are ongoing and will be presented at SETAC.

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3.25.T-02 Total Oxidizable Precursor (TOP) Assay as a Tool to Assess Transformation and Mineralization of PFAS in Sewage Sludge Treatment

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The presence of per and polyfluorinated alkyl substances (PFAS) and precursors in sewage sludge from wastewater treatment plants (WWTPs) has raised concern as it limits reuse. Knowledge gaps exist with regard to precursor transformation during traditional sludge treatment, such as anaerobic digestion (AD) and novel approaches, such as pyrolysis, and how it affects environmental impact of these processes. Therefore, the total oxidizable precursor (TOP) assay was applied to sludge samples from before and after AD from 3 WWTPs, and to 6 sludges and the 23 biochars resulting from full-scale relevant pyrolysis treatments at various conditions (500-800 °C; 5-50 min retention time (RT)). Mean target ΣPFAS37 concentrations in raw sludge (RS) ranged from 184 to 258 ng g⁻¹. RS contained the highest precursor loads as the TOP assay resulted in a 2-5 times increase in ΣPFAS37. A relative reduction of ΣPFAS37 in digested sludge (DS) compared to RS indicated transformation of precursors during AD which could indicate gaseous emissions and/or unmonitored congeners in DS. Both scenarios would lead to an underestimation of the environmental impact of the digestion process. Mean concentrations of ΣPFAS37 were 5.58±6.21 ng g⁻¹ in the 23 biochars produced. There were no significant correlations between PFAS concentrations and temperature or RT. The TOP assay indicated significant precursor presence in some sludge biochars with ΣPFAS37 >20 ng g⁻¹. Still, the mean removal effect (RE) across all conditions tested, was high for both target PFAS and precursors (>98%). The levels of residual PFAS detected in some biochar samples when using the TOP assay (>20 ng g⁻¹) warrant further assessment and demonstrates the need to consider non-target PFAS compounds when assessing destructive treatments. Although the TOP assay is a semi-quantitative method, it proved useful studying the effect of sludge treatments. However, optimization towards specific matrices is needed to produce more reliable data.

3.25.T-03 From Soil to Water: Comparing Insect Frass and NPK Fertilisers in Plant Performance and Environmental Safety

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Insect frass from *Hermetia illucens* is emerging as a circular and bio-based alternative to mineral fertilisers, yet comparative assessments focused on plant-level responses and environmental safety remains limited. This study evaluates two types of insect frass (cereal-based and olive pomace-based) against a conventional NPK regime using Integrated soil–plant assays and soil column leaching experiments. Brassica rapa was grown for two full life cycles under equivalent nitrogen inputs, and plant performance, soil biochemical functioning, and soil ecotoxicity (using *Enchytraeus crypticus* and *Folsomia candida*) were assessed. Additionally, leaching assays simulated intense

rainfall to quantify nutrient losses (NH_4^+ , NO_3^- , PO_4^{3-}) and the ecotoxicity of leachates to freshwater organisms (*Raphidocelis subcapitata*, *Lemna minor*, *Daphnia magna*) was determined.

Across soil–plant systems, frass-based fertilisation supported plant development comparably to the 100% NPK treatment. While exclusive frass applications produced slightly lower early-stage biomass, plant physiological and biochemical markers (including chlorophyll, pigment indices, sugars, and antioxidants) remained stable across all treatments, indicating an absence of metabolic stress. Frass amendments improved soil functioning by maintaining or enhancing enzyme activities related to carbon, nitrogen, and phosphorus cycles, and by promoting more balanced ammonium–nitrate dynamics. Soils reused for a second crop cycle (without additional fertilisation) still supported plant growth where frass was previously present.

Soil ecotoxicology tests revealed no adverse effects of frass on the reproduction or adult survival of *E. crypticus* or *F. candida*, demonstrating that both frass types are compatible with the functioning of the soil ecosystem. In leaching assays, frass amendments exhibited clear environmental advantages. Compared with mineral NPK, frass substantially reduced nutrient losses, with nutrients being more strongly retained in the upper soil layers due to the stabilizing influence of organic matter and chitin. Leachates from frass-treated soils caused no toxicity to algae, macrophytes, or crustaceans, while NPK leachates inhibited algal growth due to elevated nitrogen concentrations.

Frass, therefore, represents a viable, safe, and environmentally advantageous fertilisation strategy within circular and climate-smart agricultural systems.

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3.25.T-04 Sewage sludge application as biofertilizer: microbial effects and preliminary persistence assessment of quaternary ammonium biocides in soil

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Quaternary ammonium compounds (QACs) are high production volume cationic surfactants and biocides entering wastewater treatment plants after application in healthcare, consumer products, industry or agriculture. While typically > 90% of QACs are removed from wastewater, a significant fraction sorbs to sewage sludge with concentrations reaching up to 10^2 mg/kg. Consequently, high amounts of QACs can enter agricultural soils when sludge is used as biofertilizer. However, so far little is known about the fate and effects of sludge-borne QACs in soil ecosystems. QACs are aerobically degradable in surface water and sludge, but strong adsorption to organic matter and clay minerals may result in incomplete degradation and long-term persistence in soil. As QACs are broad-spectrum antimicrobials, exposure may negatively impact the diversity and function of the soil microbiome. This study therefore investigated the fate and effects of QACs in sludge-amended agricultural soil.

To this end, we investigated the dissipation and microbial community effects of structurally diverse QACs in controlled lab-scale soil microcosm experiments. QAC parent compounds were quantified by UPLC- MS/MS and effects on soil microbial communities investigated by 16S rRNA gene metabarcoding. QACs showed limited degradation after 28-day incubation in soil microcosm, indicating potential for persistence. They altered bacterial richness, evenness, and β -diversity in dose-dependent manner at exposure concentrations between 0.001-100 mg/kg. Alkyltrimethylammonium compounds induced a monotonic decrease in α -diversity, while benzalkonium compounds showed a toxicity threshold at 0.01 mg/kg soil dry weight, corresponding to realistic soil concentrations after sludge application.

Sewage sludge application is a major emission pathway for QAC biocides into agricultural soils. Our findings show that this may lead to (semi-)persistent contamination, negative effects on soil microbial diversity and altered microbial functions at realistic exposure levels. Further research should clarify the biotransformation of QACs, mobility and potential uptake of metabolites by field crops, as well as cumulative effects from repeated applications. These findings highlight the need to consider QAC input via sludge in future risk assessment to better evaluate potential impacts on soil ecosystems and human consumers.

3.25.T-05 Fate of Micropollutants in Sludge Treatment Reed Beds: Removal Efficiency, Persistence, and Risk Assessment for Agricultural Reuse

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Sludge Treatment Reed Beds (STRBs) are nature-based systems widely used for sustainable sludge management, offering effective dewatering and mineralization. However, the fate of organic micropollutants such as pharmaceuticals, pesticides, and biocides in these systems remains poorly understood, limiting safe reuse of biosolids in agriculture.

This study aimed to evaluate the removal efficiency and persistence of micropollutants in pilot-scale STRBs and assess potential ecological risks associated with land application of treated sludge.

Nine pilot beds planted with *Miscanthus × giganteus* were operated outdoors for 16 months under three aeration regimes: non-aerated control, passive aeration, and active aeration. Sludge from a municipal wastewater treatment plant was loaded every 40 days at an annual rate of 16 kilograms of dry matter per square meter.

Forty-nine micropollutants were monitored using liquid chromatography coupled with tandem mass spectrometry, and a preliminary risk assessment was performed using predicted environmental concentrations and no-effect thresholds for soil organisms.

Results showed that 43 compounds were detected at least once, with pharmaceuticals dominating. Most compounds exhibited strong removal trends: 34 were completely removed and 21 achieved approximately 99 percent reduction, regardless of aeration type. Aeration did not significantly influence removal, while environmental factors such as temperature and moisture played a greater role. Three compounds—benzalkonium chloride, carbamazepine, and propranolol—persisted or accumulated, raising concerns about long-term environmental impact. Risk assessment indicated that most compounds posed low ecological risk, but antibiotics trimethoprim, azithromycin, and erythromycin exceeded safety thresholds for soil microbes.

These findings demonstrate that STRBs can effectively mitigate micropollutant loads, supporting circular economy goals. However, persistent compounds and antibiotic-related risks require improved monitoring and risk assessment frameworks to ensure safe agricultural reuse.

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3.25.P Safe Organic Waste Circularity: Monitoring and Managing Contaminants in Valorisation Processes

3.25.P-Th099 Fate of four ¹⁴C-labelled organic trace contaminants brought in tropical and temperate agricultural soils with organic wastes

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Organic trace contaminant (OTC) fate in agricultural soils amended with organic waste products (OWP) has been widely studied under temperate conditions, whereas little to no data exist for tropical pedoclimatic contexts.

In this study we focused on the following question: what is the impact of OW, soil properties and time on the fate of various OTC in a soil/OW mix under controlled conditions? Our hypothesis for this experiment were:

- OTC properties will influence their fate in soil along the experiment, especially their log K_{ow}
- The quality of organic matter in OW and soil will influence the fate of OTC more than the quantity
- OTC extractability will decrease over time due to OW and soil properties

A 49-day microcosm incubation was conducted using 5 g of soil mixed with 150 mg of OWP spiked with 50 µL of ¹⁴C-labelled OTC: linear alkylbenzene sulfonate (LAS), phenanthrene (PHE), carbamazepine (CBZ), and azithromycin (AZI). Two soils with contrasting pedoclimatic origins, a Nitisol (Réunion Island, RUN) and a Luvisol (Yvelines, QA) and two OWP types (biosolids, BS ; pig slurry, PS) were tested. Mineralisation was

monitored via ^{13}C -CO₂ capture using lime traps. At days 0, 7, 28, and 49, microcosms were sacrificed for sequential extraction (CaCl₂ and methanol) to quantify readily and poorly available contaminant fractions. Methanol extracts were analysed by HPLC to identify potential degradation products.

Extraction yields decreased over time for all OTC except AZI, for which yields were too low for reliable interpretation, mainly due to adsorption mechanisms to plastic tubes. LAS and PHE were the only compounds that mineralised within the experimental timeframe, with mineralisation rates markedly higher in BS than in PS, and greater in RUN than in QA. CBZ displayed contrasting dynamics across soils: in RUN, the decrease in readily available CBZ corresponded to an increase in the poorly available fraction, whereas in QA this decrease was mainly associated with the formation of non-extractable residues. CBZ was also the only OTC that remained more available in RUN/OWP mixtures than in QA/OWP mixtures. HPLC analysis of CBZ methanol extracts showed that some degradation products were formed even if mineralization was very low. In QA/BS modality, the degradation product represented up to 90% of the radioactivity at day 49.

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3.25.P-Th100 Frass for grass: *Acheta domesticus* frass effects on soil functions and organisms

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Insect frass has garnered considerable attention as a sustainable, organic fertiliser and soil amendment.

Compared to traditional animal-based or mineral/chemical fertilisers, frass is a more sustainable option, being generally considered safer because it is organic, low in pathogens, and can boost soil health by adding beneficial microorganisms and chitin that support plant defences. But, as with all manures, its specific properties depend on the producing species. Therefore, this study focused on the house cricket *Acheta domesticus*, a potential manure producer (frass) that remains significantly understudied compared to other alternatives such as *Hermetia illucens*. Despite this gap, *A. domesticus* is a promising candidate capable of upcycling diverse substrates into valuable biomaterials, including frass, protein, lipids, and chitin.

Before frass can be widely used in agricultural soils, it is essential to assess both its safety for soil organisms and its influence on soil functioning. This study evaluated two aspects of frass application: (1) ecotoxicological effects on representative soil organisms and (2) soil enzymatic activities related to nutrient cycling. Ecotoxicity was assessed using the soil invertebrates *Enchytraeus crypticus* and *Folsomia candida*. Organisms were exposed to six frass concentrations in soil (0 – 1.045%), from a reference application rate of 0.261 % (3.47 tons/ha prepared according to the nitrogen needs of *Avena sativa*, equivalent to 130 kg N/ha) and an estimated frass mineralisation coefficient of 50%. Soil biochemical functioning was assessed by measuring the activities of Urease (UA), Dehydrogenase (DHA), Basic- and Acidic-phosphatase (B-, A-P), Arylsulfatase (AS), and β -glucosidase (β G) in amended LUFA 2.2 soil.

At agronomically relevant application rates, cricket frass did not induce mortality in *E. crypticus* or *F. candida*. It significantly increased the reproductive output of the former, matching the responses previously observed with other types of insect frass. Soil enzymatic activities showed no statistically significant changes within these relevant concentrations, indicating that frass did not disrupt or improve key soil biochemical processes in the short exposure time. Taken together, these results suggest that cricket frass can be safe for soil invertebrates, and importantly, it does not impair nutrient-cycling processes.

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3.25.P-Th101 Black Soldier Fly Frass from Olive Pomace as a Sustainable Soil Amendment in Cabbage Production

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Synthetic nitrogen fertilizers supply ~80% of global agriculture but generate substantial environmental costs (€35–230 billion annually in Europe). Circular bioeconomy approaches, valorizing agro-industrial waste, offer alternatives. Black soldier fly (*Hermetia illucens*, BSF) larvae efficiently bioconvert organic residues into nutrient-rich frass suitable for agricultural use. Olive pomace residue of the olive oil industry (exceeding 15 million tonnes/year globally), containing phytotoxic phenolic compounds and a low pH, presents both a waste management challenge and an opportunity for circular valorization. We assessed olive pomace and cereal-derived BSF frass as partial NPK substitutes in cabbage (*Brassica oleracea* var. *capitata*) production within a comprehensive environmental and agronomic framework. A greenhouse study (90 days, n = 5 replicates per treatment, 100 cabbages per treatment) compared six treatments: UF = unfertilized control; NPK = 100% NPK fertilizer; OP25 = 25% olive pomace-derived BSF frass + 75% NPK; OP50 = 50% olive pomace-derived BSF frass + 50% NPK; C25 = 25% cereal-derived BSF frass + 75% NPK; C50 = 50% cereal-derived BSF frass + 50% NPK. All fertilized treatments received the same amount of nitrogen (150 kg N ha⁻¹). We measured crop yield, soil biological activity (MacFayden mesofauna extraction, bait lamina feeding tests, and enzymatic activities), decomposition kinetics (Tea Bag Index), plant physiology (gas exchange and pigments), and environmental safety (*Daphnia magna* - acute toxicity on soil elutriate). An increased marketable yield (28-31%) was observed in the frass treatments compared to the unfertilized treatment, and a difference of (-3% to +9%) was noted compared to the 100% NPK treatment. Soil mesofauna was significantly higher in the treatment with 50% of BSF substitution to NPK fertilizer. Additionally, significantly higher feeding activity was observed in the BSF frass-treated samples, and no aquatic toxicity was detected in the soil elutriate. BSF frass from olive pomace and cereal substrates demonstrates promise as a partial NPK substitute, enhancing soil biological activity and ecosystem services while maintaining crop productivity and environmental safety. These findings support the use of circular bioeconomy approaches for valorizing agro-industrial residues such as olive pomace using insects as bioconverters.

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3.25.P-Th102 Assessment of Animal Manures as Sources of Microplastics and Phthalates in Agricultural Soils

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Plastics and their associated chemicals are widespread across environmental compartments. The land application of animal manures raises concerns about the contribution to microplastics (MPs) and plastic-derived chemicals such as phthalates contamination in soils. Once present, these contaminants may persist, interact with soil biota, and enter food and water pathways, posing risks to environmental and human health. Nevertheless, the sources, behaviour and fate of MPs and phthalates introduced to agricultural environments via organic amendments remain poorly characterised.

This study investigates manures from bovine, ovine, chicken, duck, and pig production systems in Ireland to assess their potential contribution to MPs and phthalates in agricultural soils. Representative manure samples were collected from farms across the country. The research is currently in its method development phase, focusing on analytical techniques for the extraction, isolation, and quantification of MPs and phthalates. This includes optimisation of organic digestion and filtration methods for MPs and solvent extraction, followed by gas chromatography–mass spectrometry for phthalates. Once validated, these methods will be applied to characterise and quantify MPs and phthalates in the collected manure samples

The outcomes will provide baseline data on the occurrence and variability of MPs and phthalates in different manure types and offer insight into their potential contribution to soil contamination and subsequent exposure pathways within an Irish context. This work will contribute to a better understanding of how agricultural recycling of organic materials may act as a vector for plastic-associated pollutants and influence human

exposure through the soil–food–water nexus. The findings will inform future risk assessments and support the development of mitigation strategies for sustainable agricultural practices.

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3.25.P-Th103 Assessing Metal Risks in Circular Insect Farming Using a Toxicokinetic Framework

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Under the Novel Food Regulation (EU) 2015/2283, the yellow mealworm (*Tenebrio molitor*) is one of the four insect species currently approved for human consumption in the European Union. Its ability to grow on diverse organic waste streams supports circular food production, but also raises questions regarding contaminant transfer and safety. Although metal accumulation has been reported in edible insects, most studies rely on single-endpoint measurements, providing limited insight into how contaminants actually behave within insect tissues. A toxicokinetic (TK) approach is therefore essential to determine whether accumulation is temporary or persists despite depuration. To address this gap, we quantified the uptake and elimination kinetics of cadmium (Cd), arsenic (As), and lead (Pb) in *T. molitor* larvae. Organisms were fed metal-spiked semolina wheat for a period of 21 days (uptake phase), followed by a 21-day on unspiked food (elimination phase). Control groups were fed clean substrate throughout the experiment. Targeted exposure concentrations matched EU feed limits (Directive 2002/32/EC): 2 mg Cd/kg, 2 mg As/kg, and 10 mg/Pbkg. Organisms were sampled on days 0, 1, 3, 7, 14, 21, 22, 24, 28, 36, and 42; lyophilised, and their metal content was then analysed using inductively coupled plasma mass spectrometry. Accumulation in *T. molitor* was metal-specific. At the last day of exposure (day 21), Cd (2.6 ± 0.3) g Cd/kg; bioaccumulation factor [BAF]=2.03 and As (7.03 ± 1.05 g As/kg; BAF=32.82) exceeded the regulatory limits for feed in the EU after exposure. In contrast, Pb accumulated to a lesser extent (2 ± 0.62 mg Pb/kg; BAF=0.24). Cd was quickly eliminated and reached internal levels in larvae below the threshold after 1 day in the clean substrate, with half of the compound (DT50) being eliminated in 6.87 days. In turn, internal concentrations of As exceeded EU feed limits during the entire experiment, revealing a DT50 value of 94 days, whereas accumulated Pb was promptly eliminated (DT50=2.12 days). These findings suggest that safety cannot be inferred from exposure levels alone and must also account for depuration dynamics. TK parameters provide a predictive tool for regulatory decision-making by highlighting contaminants retained in insects after depuration. Integrating TK models into edible insect safety procedures is thus crucial to ensure that circular production systems are not only sustainable, but safe.

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3.25.P-Th104 From Printed Disposable Wearable Electronics to Waste: Oxidation, Microbial Inhibition, and Environmental Impact of Copper, Silver, and Carbon Inks on Paper Substrates by Life Cycle Analysis

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In the Sustronics project, funded by the EU, a smart wound dressing based on printed electronics has been developed that integrates a disposable electronic sensor built on sustainable substrates and comprising more sustainable electronic materials. The dressing is designed to electronically monitor pH and temperature to flag infection, enabling timely therapy adjustment and supporting improved patient outcomes . Flexible printed electronics based on printable inks on sustainable substrate materials like paper and biobased

plastics have several advantages over the conventional electronic technology which applies rigid FR4 boards and subtractive technology with considerable environmental impact.

Flexible printed electronics are expected to be more frequently integrated into products like clothing, health care wearable and consumer products. Therefore, these materials will increasingly enter hospital waste streams and municipal waste streams, with potential contact with nature through applications of bottom ash from waste incineration or via landfilling. This paper will use a biodegradation study of electronics in compost comprising Cu, Ag and Carbon particles on a paper substrate to apply first insights into exposure of nature to these new material combinations and provide insights to the LCA study of the End-of-Life Scenario. The metals showed inhibition in biodegradation of the substrate in contrast to carbon and more severely for the Cu filled material. Analysis of the compost after the biodegradation experiment suggested that the difference in inhibition is caused amongst others by the oxidation potential of the metals: less noble metals oxidise more readily and after being leached out into the compost inhibit microbes. These findings provide first insights into exposure risks from emerging material combinations in flexible electronics.

Modelling the end of life scenario's by LCA is presented but the background information for this phase of the life cycle is limited. The results underscore the need for dedicated inventory data and impact characterization tailored to printed electronics, and indicate that material selection (e.g., favoring carbon-based conductors over Cu) can reduce potential environmental burdens at end of life. This work contributes initial empirical evidence and methodological input to support future LCA of flexible electronics, and aims to steer the development of materials and designs that are more compatible with natural systems.

Disclaimer/Disclosure:

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3.25.P-Th105 Emerging Pollutants in Walloon Sewage Sludge: Towards an Integrated Methodology to Evaluate Health and Environmental Impacts of Farmland Spreading

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In Wallonia (Belgium), farmland spreading is the main solution for sludge management. Prior to land application, regulatory quality control covers only a few parameters, including common organic pollutants. However, emerging substances in sewage sludge have been poorly explored to date.

The main objective of the study was to assess the impact of sludge farmland spreading on human health and the environment, with a focus on some endocrine disruptors (EDCs) and pharmaceutical products. Following a prioritization of relevant substances and a selection of Walloon Waste Water Treatment Plants (WWTPs), chemical and ecotoxicological characterization of sludges was carried on. Finally, a health risk was assessed. The aim of the prioritization was to highlight the substances likely to be found in sludges and potentially having the highest hazards for humans and the environment. The methodology was based on physico-chemical properties and regulatory criteria. Presence in Walloon sludges was then confirmed using high-resolution chromatography techniques, and a specific chemical characterization was carried out. At the same time, an ecotoxicological characterization assessed the intrinsic toxicity of raw sludge and the effect of sludge mixed with soil using a battery of tests. The scope of this battery meant that all aspects are covered: terrestrial and aquatic ecosystems, different trophic groups, complementary forms of toxicity, direct and indirect exposure routes. In addition, the endocrine disruption potential was characterized using in vitro YES/YAS tests. Finally, the assessment of the health risks associated with the use of WWTP sludge in agriculture was conducted by modelling exposure doses on the basis of the chemical results.

In conclusions, prioritization step sets up a list of 69 substances, and 29 representative WWTPs with authorisation to spread the sludge in Wallonia. The specific chemical characterization was carried out based on 160 sludges. The EDCs analyzed belong mainly to the chemical families of alkylphenols, chlorophenols, phthalates, perfluoroalkyl compounds (PFAS) and personal care products. A general frequency of 36% was observed with two EDCs over 95%. The ecotoxicological characterization on 29 sludges shows six sludges with high effects (indirect and direct exposure). And finally, based on some assumptions, results of the risk assessment show that a risk associated with PFOS and PFOA in WWTP sludge farmland spreading cannot be excluded.

3.25.P-Th106 Risk Assessment of Organic Contaminants in Sewage Sludge Applied to Agricultural Soil
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The Norwegian Scientific Committee for Food and Environment (VKM) has on request from The Norwegian Food Safety Authority (NFSA) performed a risk assessment of organic contaminants in sewage sludge. Norway is an elongated country where climate precipitation and temperature vary highly, and thus also agricultural cropping systems and practice. Five municipalities/regions across North, South, East, and West were chosen to capture climate and agricultural differences and thus used in the risk assessment.

In the present risk assessment NFSA asked for evaluation of many scenarios including today's common sludge application according to today's the Norwegian fertiliser regulation in terms of amount of sewage sludge, application time before cultivation or harvesting. In addition, it was asked for an evaluation of new ways of application of sewage sludge and of sewage sludge-based products as biochar, ash and struvite and some other sludge-based products. Application methods (effect on soil depth and soil mixing or not), and shorter period from application before cultivation and harvesting, were also included.

A science-based risk assessment was conducted, consisting of the four steps: i) identifying hazards (measured data), ii) description and characterisation of the hazards (from literature), iii) assessment of exposure/intake of the hazard (modelling), and iv) description/characterization of risk (Comparing exposure to toxic effect level). Target organisms in the risk assessment included environmental living organisms in water and soil, farm animals – including grazing animals, and humans. Due to lack of knowledge, risk towards farm animals and humans were not a proper risk assessment but an evaluation of likelihood for increased exposure over years of sewage sludge application (up to 100 years). In addition, for selected contaminants terrestrial secondary poisoning of mammals and bioaccumulation in fish and potential transfer to humans and up in the food web were evaluated.

The number of organic contaminants in sewage sludge is large, around 1000 different compounds have been analysed for in Norwegian sewage sludge, and a prioritisation of contaminants were performed, following same approach as JRCs screening for organic contaminants in wastewater from 2022.

Highlights from the risk assessment will be presented, as well as some urgent knowledge gaps in order to develop robust and reliable risk assessments.

3.25.P-Th107 The impact of pyrolytic conversion and biochar amendments on metal exposure and mobility in soil

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Over the last two decades, pyrolytic conversion and biochar amendments have emerged as promising technology within waste management, resource recovery and contaminant remediation. More recently, large scale biochar amendments have been suggested as an important pillar for carbon capture within the green transition of Danish Agriculture, which has triggered concerns and discussions about the associated contamination risks. This concern is particularly relevant for metals, which are known to up-concentrate in biochar. However, pyrolysis also promotes the formation of mineral phases which enhances metal retention in the solid phase, thereby reducing aqueous metal concentrations and hence metal mobility. These changes would then be reflected in a higher solid–water distribution coefficients (KD values). This raises critical questions regarding the environmental fate of metals in biochar-amended soils, including their mobility, leaching potential, and long-term impacts on soil and water quality.

This study addresses these concerns by investigating the behavior of metals in biochar, with a focus on their exposure and mobility under varying pH conditions. Carefully paired samples from wastewater treatment plant (WWTP) sludge and biogas residue fiber, along with their corresponding biochars, were obtained from full-scale pyrolysis plants in Denmark. Aqueous-phase metal concentrations in equilibrium with the feedstocks and their derived biochars were analyzed, covering a wide range of elements, including rare earth elements (REEs). To further assess metal partitioning between solid and aqueous phases, solid–water distribution coefficients were determined with two different approaches. Finally, column experiments were conducted to determine the

metal leaching from soils at varying amendments of biochar from straw, biogas residue fiber and WWTP sludge.

3.25.P-Th108 Reduced Leaching of PFAS and Polar Organics from Biochar: The Combined Effect of Pyrolytic Removal and Increased Sorption

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The Danish Government has presently a strong focus on pyrolysis as a key technology in the green transition in Denmark. This is due to its potential and increasing applications within circular economy, carbon sequestration, soil improvement, and contaminant removal. Sewage sludge (SS) from wastewater treatment plants (WWTPs) and biogas residue fiber (BRF) from biogas production offer high nutrient content that makes them promising candidates as fertilizers. However, the presence of heavy metals and organic pollutants such as per- and polyfluoroalkyl substances (PFAS) in the feedstock or contaminants produced during pyrolysis, has raised concerns regarding its safe utilization as biochar fertilizer. The critical question is therefore: How does the pyrolytic conversion of feedstock to biochar (BC) affect the exposure, mobility and potential impact of pollutants in the environment?

The first aim of this study is to determine the overall effect of the pyrolytic conversion of WWTP sludge and biogas residue fibers to biochar on the leachability, exposure and mobility of PFAS and polar organic contaminants. We hypothesize that pyrolysis substantially decreases aqueous concentrations for most if not all organic contaminants due to (1) pyrolytic removal and (2) increased matrix to water distribution coefficients. Dewatered SS and BRF were used as model feedstocks, with carefully matched samples of feedstock and BC obtained from different full-scale pyrolysis plants in Denmark. Equilibrium batch tests with feedstock and corresponding BC suspended in water were conducted, followed by up-flow column percolation tests with BC and soil mixtures. Aqueous concentrations from both experiments were measured across a broad range of polar organic chemicals including PFAS, utilizing advanced analytical techniques such as LC-HRMS. To comprehensively assess the impact of pyrolytic conversion on various pollutants, non-target analysis was conducted in addition to targeted PFAS analysis. The results from the batch equilibrium tests show exposure and leachability reductions of polar organic contaminants in BC relative to the corresponding feedstocks. The outcomes of this study will inform regulators on the potential environmental impact of biochar application in agriculture.

Disclaimer/Disclosure:

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3.26 Soil–Plant Transport and Mineral Weathering: Spatiotemporal Environmental Implications

3.26.T-01 The Effects of Ageing and Heterogeneous Distribution of Perfluoroalkyl Acid (PFAA) in Soil on Their Uptake by Allotment Vegetables

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Home-grown vegetables are a known pathway for human exposure to per- and polyfluoroalkyl substances (PFASs). Although bioconcentration factors (BCFs; -) are available to relate soil contamination to plant concentrations, these values are often deduced under laboratory conditions and fail to take into account key environmental factors from the field. With PFAS contaminations often being decades old and concentrated in the upper part of the soil, this study assessed the effect of the ageing and heterogeneous distribution of the most widespread perfluoroalkyl acids (PFAAs) on their BCF in common allotment vegetables. To study ageing, a small pot trial was set up in which ¹³C-labeled PFAA components were added to three historically contaminated soils, on which celery and radish were planted. Results showed significantly higher BCF values for the ¹³C spiked compared to ¹²C aged PFAAs, with values decreasing with increasing chain length, irrespective of the contaminants' suspected age. To study the effect of heterogeneous PFAA distribution, the lower part of PVC pots (30-40 cm in height) was filled with uncontaminated soil, while historically contaminated soils were used for the upper part. Besides the aforementioned vegetables, also lettuce and hemp

were planted, the latter for its phytoremediation potential. While lettuce and celery have a shallow fibrous root system, radish and hemp have a deeper taproot system. This experiment is ongoing and results will be communicated at the conference. We speculate that root distribution critically explains the plant-specific BCFs in this setup. This study is anticipated to lead to more field-relevant BCF values than those based on spiked and homogenized soils.

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3.26.T-02 A Novel Workflow to Identify the Environmental Fate of Emerging Contaminants in the Wastewater – Soil – Plant Continuum

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The reuse of treated wastewater (TWW) for irrigation represents an important pathway through which emerging contaminants enter agroecosystems. Although environmental monitoring campaigns continue to expand, their success remains constrained by the performance and selectivity of multi-residue analytical techniques, which routinely overlook lesser-known chemicals in terrestrial matrices. Advances in environmental fate modelling and non-targeted screening (NTS) offer new opportunities to characterise contaminant behaviour across the wastewater–soil–plant continuum; however, the high cost, time demands, and methodological limitations of NTS restrict its widespread application. Here, we present a validated modelling framework and an accompanying Python workflow that integrates NTS data from wastewater treatment plant influents and effluents with advanced fate modelling tools. Model evaluation using targeted datasets (n = 69 chemicals; 9 Zea mays fields; 12 Solanum lycopersicum fields) demonstrated strong predictive performance, with 90.5% and 89.2% of measured concentrations meeting “good match” criteria for maize and tomato fruits/grains, respectively. When semi-quantified TWW concentrations were used to simulate PEC_{grain}, 88.89% of predicted values for Zea mays grains fell within one order of magnitude of semi-quantified measurements, including 17β-estradiol, carbamazepine, di(2-ethylhexyl) phthalate, dodecyl sulphate, N,N-diethyldodecanamide, N-phenyl-1-naphthylamine, octyl hydrogen phthalate, and venlafaxine. Model simulations further identified high-priority contaminants within plant tissues. In maize leaves, the top five emerging contaminants were 4-nitrophenol > fexofenadine > atenolol > octyl hydrogen phthalate > lidocaine, with a similar ranking observed in grains alongside PPG n4, 4-formylaminoantipyrine, carbamazepine, N-phenyl-1-naphthylamine, and tris(2-butoxyethyl) phosphate. Pharmaceuticals were prevalent across TWW and plant matrices, with compounds such as estriol, carbamazepine, atenolol, 11-ketotestosterone, flecainide, trenbolone, atomoxetine, climbazole, and artemotil detected in both leaves and grains. Collectively, these results demonstrate that coupling NTS outputs with mechanistic modelling offers a scalable and transferable approach for predicting contaminant fate under real-world irrigation scenarios, while revealing overlooked chemicals of emerging concern in agroecosystems.

3.26.T-03 Quantifying the Impact of Plant Uptake on Watershed-Scale Pesticide Concentration in Flowing Water Bodies

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SWAT (Soil and Water Assessment Tool) is a widely used tool for modeling pesticide transport through agricultural watersheds. It is, however, currently lacking plant uptake functionality, although plants absorb chemicals from the soil, particularly persistent compounds that remain in the root zone due to slower soil degradation or reduced mobility. This represents a significant gap in simulating chemical fate processes, as plant uptake can remove substantial amounts of pesticides from soil systems during growing seasons. In this work we updated the SWAT code to include plant uptake, building on recent improvements in the representation of subsurface chemical transport pathways including tile drain and groundwater flow. Our approach calculates uptake based on plant water consumption patterns, compound-specific resistance terms, and chemical concentrations in soil water. The implementation ensures that uptake occurs only during active plant growth periods and follows established water uptake distributions with depth. We tested the updated model in two European agricultural watersheds using a stable pesticide transformation product that is taken up by plants according to laboratory-derived uptake factors. Incorporating plant uptake reduced predicted concentrations of

the simulated metabolite in streamflow by 5 to 17%, with differences between catchments attributed to variations in agricultural land use intensity and cropping patterns. This indicates that plant uptake is an important removal pathway for slowly degrading compounds and provides improved capability for evaluating farming practices and mitigation strategies. The enhanced model demonstrates very good performance while providing more mechanistically complete representation of chemical fate processes. We provide the new functionality for both SWAT2012 and SWAT+, enabling watershed managers and researchers to better assess pesticide environmental fate in agricultural systems.

3.26.T-04 Integration of Prospective Environmental and Economic Impact Assessment for Emergent Mineral Carbonation of Steel Slags Process Design in Agriculture

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Emerging technologies are often characterized by low Technology Readiness Levels (TRLs), which present challenges such as limited life cycle inventory data, high uncertainty in available datasets, and unknown economy-of-scale effects. These factors complicate the assessment of environmental and economic impacts. However, low TRL stages also offer opportunities for design flexibility and performance optimization, enabling improvements in both technological efficacy and sustainability outcomes compared to mature technologies. This study proposes an integrated framework for prospective environmental and economic assessment of emerging technologies. The framework combines Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) with Multi-Criteria Decision Analysis (MCDA) using the Analytical Hierarchy Process (AHP) to evaluate trade-offs and identify optimal scenarios. Prospective LCA (pLCA) is employed as a scenario analysis tool to determine key technological parameters influencing global warming potential (GWP) and overall environmental performance. The approach is demonstrated through a case study on mineral carbonation of steel slags as a carbon capture, utilization, and storage (CCUS) technology. Results identify optimal carbonation conditions based on environmental and economic criteria, estimate ideal process durations, and highlight carbonation efficiency as a critical parameter for reducing GWP. This framework provides actionable insights for guiding the development of emerging technologies toward environmentally and economically sustainable pathways.

3.26.T-05 Fate of Pharmaceuticals and Human Health Risk Assessment in Agricultural Water Reuse: Case Studies in Greenhouse and Soil Cultivation.

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Water scarcity in agriculture is increasing due to changing climate conditions, driving interest in reclaimed water as an alternative irrigation source. The LIFE ACLIMA project demonstrates climate-adaptive solutions for horticulture and agriculture in Flanders, including reuse of municipal wastewater treatment plant (WWTP) effluent. EU Regulation 2020/741 sets minimum requirements for agricultural water reuse regarding e.g. pathogens, but micropollutants such as pharmaceuticals lack target values and require risk-based evaluation. In Flanders, the Decision of the Flemish Government provides detailed guidance for environmental risk assessment of micropollutants. However, human health risk assessment (HHRA) remains undefined, creating uncertainty and acting as a barrier to circular water adoption in agriculture. The LIFE ACLIMA project addresses this gap by evaluating the fate of pharmaceuticals and associated human health risks in two effluent-irrigated systems: (i) greenhouse bell pepper cultivation and (ii) open-field soil cultivation. Pharmaceuticals were detected in irrigation water and, at low concentrations, in greenhouse fruits and leaves. Soil trials as well confirmed pharmaceutical uptake in the fruits in low concentrations. HHRA was performed by estimating daily intake (EDI) from measured crop concentrations and comparing it to acceptable daily intake (ADI). To ensure a precautionary approach, multiple ADI values are generated based on various thresholds and the most conservative ADI values were applied in all calculations. Under these assumptions, consumer exposure remained well below thresholds of concern, indicating negligible health risk for the tested scenarios. These findings provide critical evidence for regulatory frameworks and risk communication. While pharmaceuticals can enter the food chain through reclaimed water irrigation, current data suggest minimal human health risk when conservative assumptions are used. Further research on additional crops, long-term soil dynamics, and cumulative exposure is recommended to strengthen confidence in safe water reuse practices.

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3.26.P Soil–Plant Transport and Mineral Weathering: Spatiotemporal Environmental Implications

3.26.P-Th109 Assessment of Organic Contaminant Uptake in Field-Grown Fruits Irrigated with Recycled Water and Amended with Class A Biosolids

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As pressures on freshwater resources intensify, recycled water and Class A biosolids are gaining recognition as valuable inputs for sustainable agriculture, offering agronomic benefits such as improved soil quality, enhanced plant growth, and nutrient recycling. However, both recycled water and biosolids can contain trace concentrations of unregulated organic chemicals (UOCs), including pharmaceuticals, personal care products, plasticizers, flame retardants, and per- and polyfluoroalkyl substances. Despite growing interest in repurposing these waste-derived materials, data on plant accumulation of UOCs and subsequent human dietary exposure remain limited, particularly under field conditions.

To address these knowledge gaps, a large-scale field study was conducted in semi-arid Southern California using integrated recycled-water irrigation and biosolids fertilization. Four staple fruit crops, i.e., apple, avocado, mandarin, and peach, were grown using standard agronomic practices, with tertiary treated wastewater as the sole irrigation water source and Class A biosolids partially or fully replacing synthetic fertilizers. A list of 24 UOCs were targeted for analysis using UPLC-ESI-MS.

In our previous study on vegetable crops, 20 UOCs were detected at concentrations from 0.93 to 455 ng/g dry weight (d.w.). Dietary intake estimates for these UOCs, based on U.S. EPA reference consumption rates, suggested an adult annual intake of 4880 µg, equivalent to < 0.2 µg/kg body weight per day for a 70 kg person. Compared with available acute toxicity data, these exposures are several orders of magnitude below levels known to cause adverse effects. We therefore hypothesize that concentrations in fruit trees, given their greater biomass and longer growing season, will be even lower, supporting the sustainable reuse of large volumes of biosolids.

These findings provide field-based insights into the environmental behavior and food-chain transfer potential of UOCs in agricultural systems, suggesting that treated municipal wastewater and biosolids can be safely integrated into food production within a circular economy framework. Future work will expand these investigations to long-term and multi-season trials that assess cumulative effects of repeated applications. Collectively, these studies will inform human health and ecological risk assessments and support best management practices for the responsible and sustainable reuse of treated waste-derived resources in agriculture.

3.26.P-Th110 Environmental Distribution and Soil-Plant Transfer of Carbon Based Nanomaterials

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Carbon-based nanomaterials (CBNMs), such as carbon nanotubes (CNTs) and fullerenes, are widely used in industrial products, textiles, coatings, batteries, and electronics. Their growing use has increased their release into the environment, with untreated wastewater contributing up to 99% of CNT emissions. Environmental fate studies also show that soils receive the largest share of these materials (about 91.2%), followed by freshwater systems (about 2.8%). Understanding how CBNMs accumulate in soil and how they move into edible plants is therefore important for assessing human exposure and potential health risks.

In this study, concentrations of CNTs and fullerenes in soils and sludge-treated soils were collected from the literature to develop realistic exposure ranges. The data were log transformed and analyzed using the mean, standard deviation, standard error, and two-tailed 90% confidence intervals (CI), which were later back-transformed to original units. CNT concentrations were consistently higher than those of fullerenes in both soil types. In sludge-treated soils, CNTs had a mean concentration of 59.13 ng/kg (90% CI: 37.35–93.63), which was about 18 times higher than fullerenes (3.29 ng/kg; 90% CI: 1.12–9.64). In natural soils, CNT concentrations (1.88 ng/kg; 90% CI: 0.99–3.58) were around 31 times higher than fullerene levels (0.06 ng/kg; 90% CI: 0.025–0.142), indicating that CNTs are more persistent in the environment.

Root–shoot transfer factors (TFs) were compiled across plant species to assess internal mobility. CNTs exhibited very low transfer, with TF values ranging from 0 to 0.10, whereas fullerenes showed significantly

higher mobility, with TF values ranging from 0.049 to 1.156. The highest fullerene TF was roughly 12 times greater than the maximum CNT TF, and the average fullerene mobility was 10-15 times higher. Overall, CNTs tend to remain in soil for longer periods, while fullerenes move more easily within plant tissues. These contrasting behaviors highlight the need for more accurate exposure assessments of CBNMs in agricultural environments.

3.26.P-Th111 Refining The Hydroponic Method For Determining Plant Root Uptake Of Chemicals: An Evolved Ring Test Design

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Uptake of soil chemicals into plants critically shapes environmental fate and is quantified in models like PEARL and PELMO via the Transpiration Stream Concentration Factor (TSCF). Hydroponic studies isolate root uptake from soil effects (sorption, degradation), revealing a compound's intrinsic uptake potential. An earlier OECD round robin test using a prior design showed substantial inter-laboratory variability. Building on those findings, the CLE/IVA Working Group "Plant Uptake" developed an evolved hydroponic protocol to target key sources of variation.

Key refinements to reduce variability

- Growth stage standardization: Plant age is replaced with BBCH growth stages (e.g., BBCH 12–13 and 21–24) to improve physiological uniformity across labs.
- Environmental control: Light is specified by PPFD within 150–550 $\mu\text{mol}/\text{m}^2/\text{s}$, a biologically relevant metric, instead of illuminance (Lux).
- Chemical/analytical precision: Nutrient solution pH tightened to 5.5–6.0; mass balance recovery constrained to 95–105%.

Validation via ring test A five-laboratory inter-laboratory ring test was initiated using 1,2,4-triazole as the test item. Preliminary results from one lab show excellent intra-laboratory precision:

- BBCH 11–12: Mean TSCF ? 0.46, 0.47, 0.43 at 0.1, 1, and 10 $\mu\text{mol}/\text{L}$.
- BBCH 21–24: Mean TSCF ? 0.57, 0.58, 0.55 at the same concentrations.

Early data suggest the refined protocol produces robust, reproducible TSCF measurements across concentrations and growth stages. The forthcoming full presentation will deliver a comprehensive statistical analysis from all five laboratories, quantify improvements in inter-laboratory reproducibility relative to the previous study, and provide a formal validation of the evolved test design.

Disclaimer/Disclosure:

Competing interests

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Employment

Maximilian Küppers and Marc Lamshöft report a relationship with Bayer AG Division Crop Science, Monheim, Germany that includes employment.

3.26.P-Th112 Upward Movement of Dimethylsilanediol (DMSD) in Soil-Plant Systems

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Methylsiloxanes, both polymeric and oligomeric, are widely used in consumer and industrial products. Their environmental degradation, particularly following land application of wastewater sludge, leads to the formation of smaller degradation products. Among these, dimethylsilanediol (DMSD) is a key monomeric hydrolysis product. Although DMSD is known or expected to occur in soil, its transport behavior has yet to be comprehensively characterized. To address this, we developed a dynamic model to estimate DMSD formation and movement under varying weather scenarios. Model parameters were optimized using experimental data

from bench-scale soil column studies. In these experiments, octamethylcyclotetrasiloxane (D4), a parent compound, was spiked into the topsoil to simulate realistic conditions and track DMSD behavior. The model confirms that DMSD generation from D4 increases as soil moisture decreases and that DMSD migrates upward with water and volatilizes at the soil surface during dry conditions. It also enters plant roots and translocates through the stem to the leaves, facilitated by its high water solubility and low affinity for plant tissues. Unlike typical soluble pesticides found in agricultural soils, DMSD can volatilize from leaf surfaces without accumulating in the plant, due to its relatively low KOA value. These results suggest that DMSD primarily moves upward in soil and plants, with volatilization to air being a dominant pathway. Downward transport toward groundwater appears unlikely. Understanding this behavior is essential for assessing environmental exposure and potential risks associated with siloxane degradation products.

3.26.P-Th113 Four years of monitoring the behavior of micropollutants whose source is treated wastewater or sludge from wastewater treatment plants in the soil-water-plant system

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To assess the behavior of selected micropollutants in the soil-water-plant system, nine raised beds were installed in March 2021 at the wastewater treatment plant for ?eské Bud?jovice WWTP in the Czech Republic. Two beds contain soil taken from the surface horizon of the Arenic Regosol and seven beds contain soil from the surface horizon of the Eutric Cambisol. Several scenarios were implemented: A. irrigation with drinking water (control); B. irrigation with treated wastewater; C. application of treated sewage sludge; and D. application of composted sewage sludge. In 2021, either corn or a mixture of different types of vegetables (lettuce, onions, carrots) was grown on the beds. Results from this year have been published by Kodešová et al. (<https://doi.org/10.1016/j.scitotenv.2023.167965>). The experiment continues and other types of crops are grown in the beds. Both biosolids are applied every year before planting or sowing plants. Since 2022, the beds with composted sewage sludge have been additionally irrigated with treated wastewater, and since 2023, the beds have been roofed so that the behavior of micropollutants is not affected by rainwater. Initially, attention was paid primarily to pharmaceuticals, and later other substances were included in the monitoring, such as those found in tires, surfactants, dyes, fire-fighting foams, disinfectants and cleaning products. In general, compounds of high sorption and high stability in the environment were accumulated in the soils. Mobile compounds were found in the water drained from the beds, taken up by plants and accumulated in the leaves. Less mobile compounds remained mainly in the underground parts of the plants. However, it was also found that some compounds, despite being highly sorbed in the soil environment and having a low potential to be transported in plants due to their properties, were accumulated in the leaves or were leached from the beds. Compost reduced the leaching and uptake of compounds from wastewater. After the roof was installed, the concentrations of substances in the monitored system increased and more compounds were quantified than in the first two years.

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3.26.P-Th114 Dynamic Modeling of Pesticide Uptake From Coated Seeds Into Maize Plants and Translocation to Pollen

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Agricultural insecticides are often applied via seed coating. The resulting chemical residues can potentially impact pollinating insects, an important aspect currently not included in evaluating the environmental performance of product and technology life cycles. Here, we propose a dynamic mechanistic model for chemical

plant uptake from soil and translocation to nectar and pollen, for simulating residue concentrations in soil and plant parts as a function of time. Modeling results were compared with concentrations of different insecticides observed in field experiments with seed (or soil) and spray application.

Observed concentrations could be explained well by modeling. Highest concentrations in stem, leaves, flowers and thus also pollen were seen during times of intense growth and small plant mass: at the beginning of the exponential growth phase, when intense fluxes by transpiration and assimilate stream meet small compartment volumes. Degradation rates were calibrated with the model, which exceeded dissipation rates directly fitted to measured concentrations. This can be explained by the continuous delivery of chemical mass predicted by the model due to translocation from roots via stem to leaves, flowers and pollen, which cannot be considered by empirical methods. Simulated dissipation encompasses growth dilution, degradation and (if relevant) volatilization with half-lives of 0.17-0.53 days for pollen, close to half-lives observed for pesticides applied via spraying. In the simulated field tests, 1 mg imidacloprid was applied per seed (11.9-13.5 mg/m²) with resulting pollen concentrations of 0.001-0.012 mg/kg fresh weight during the pollen shed period. Corresponding RUD (residue per unit dose) values are 0.0083-0.1 mg/kg per kg applied on one ha, which is low compared to the median RUD reported for spray application (26 mg/kg). Hence, seed coating is an effective application method for insecticides, but the drawback is the presence of toxic residues during the whole growth period, endangering visiting insects, such as pollinators, which can potentially be exposed.

In conclusion, our model predicts translocation of mobile, persistent and non-volatile chemicals from soil to pollen. This holds also for PMT (persistent, mobile, toxic) compounds present in reclaimed wastewater for irrigation and in sewage sludge applied to fields. Our model can be incorporated into existing frameworks to consider exposure and life cycle impacts on pollinating insects.

Disclaimer/Disclosure:

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3.26.P-Th115 Plant uptake of Organic Chemicals from Sewage Sludge applied to Norwegian Fields – Part of a Risk Assessment

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The Norwegian authorities commissioned a risk assessment of organic micropollutants in sewage sludge applied on agricultural fields. To this end, results from chemical analysis of sludge from the Norwegian sewage treatment plants were collected, together with soil properties and crop rotation data for Norwegian regions. Chemical data were downloaded from CompTox, additional physico-chemical properties were estimated with ACD. The median value of the 90%-percentiles of the measured sludge concentrations at individual sludge facilities divided by PNEC soil was employed to pre-select and reduce the number of chemicals to 301. This includes 95 medical drugs, 47 chemicals in cosmetics (among them siloxanes and benzalkoniumchlorides), 16 flame retardants (hereof 7 polybrominated), 14 PAH, 20 pesticides, 13 per- and polyfluorinated compounds, 16 plasticisers and 29 other chemicals added to plastic, six rubber chemicals, 17 surfactants, plus fragrances, solvents, antioxidants, coatings, corrosion inhibitors and others.

K_d and K_{oc} were determined for typical soils from five regions. Measured values were preferred, else, regressions (TGD for neutral chemicals, Franco and Trapp for acids and bases) were used. Bioconcentration factors in carrots, potatoes, grass and cereals were calculated with crop-specific plant uptake models. Concentrations in soil are calculated following the approach in TGD, but modified for Norwegian scenarios. Biodegradation rates are estimated with Biowin, unless measured high-quality data are found in literature. Results were sorted for bioconcentration factors (BCF), and for (preliminary) concentrations in crops. These preliminary results indicate that many chemicals well known from field studies rank high in the list of plant uptake, among them carbamazepine, caprolactam, triclosan, TCPP, TBEP, acetylsalicylic acid, benzalkoniumchlorides and fluorinated compounds. All top 100 chemicals with highest BCF have K_{oc}-values below 1000 L/kg (M, mobile), and many below 100 L/kg (vM, very mobile). However, also the very lipophilic chemicals, such as PAH, PBDE and siloxanes can pose a risk due to soil attachment and grazing animals. Calculated concentrations in crops will further be processed to calculate risks for humans, farm animals and wildlife under various scenarios of sludge application. The results will serve to decide about permitted application schemes in Norway.

3.26.P-Th116 Can Oil (Palm) and Water Mix? Ecological Risk of Heavy Metals in an Oil Palm Plantation
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The high yield potential of oil palm, alongside ever-increasing demand for palm oil derivatives, drives frequent agrochemical use to maximise productivity. Agrochemicals, such as fertilisers, can contain impurities such as heavy metals. These unintentional releases of heavy metals can cause toxic effects in organisms and pose a threat to health of the wider ecosystem. With over 30 million hectares planted with oil palm globally, and ongoing expansion in many places, understanding the ecological risk of such pollutants is critical knowledge that will underpin future management strategies to promote sustainable production. In this study, an ecological risk assessment was performed for aquatic species in water bodies receiving inputs from an oil palm estate in Sabah, Malaysia. Concentrations of 11 metals were measured in a total of 86 water samples collected from subsidiary drains (within planted blocks), boundary drains (surrounding planted blocks), and nearby tributaries. Predicted No Effect Concentrations (PNECs) for freshwater organisms were extracted from the Norman Network Ecotoxicology Database of lowest PNECs. The total dissolved concentrations of trace elements were then compared to PNECs to assess risk using a risk quotient approach. A risk quotient >1 indicates that aquatic organisms may be at risk of toxic effects. Across these 11 metals, risk quotients ranged from <0.0001 (seen for molybdenum) suggesting negligible risk, to 138.7 (seen for cobalt) suggesting high risk. Notably, As, Co, Cu, Ni and Zn showed risk quotients >1 for 44%, 67%, 59%, 80% and 52% of samples respectively. This suggests possible risk of aquatic toxicity from multiple metals in oil palm influenced waterbodies. Furthermore, the trend in risk for the majority of elements followed the pattern: subsidiary drains > boundary drains > tributaries likely due to a combination of proximity to sites of agrochemical application and dilution. This study presents a risk assessment of heavy metals in waterbodies of oil palm plantations, revealing that metals may pose a risk to aquatic organisms. Future work should investigate how metal translocation and risk varies over time as well as the direct contribution of agrochemical use to dissolved metal concentrations. Finally, the PNEC values used here are for temperate species, therefore, the relative sensitivity of analogous native species is needed to account for sensitivity differences and determine real world risk in these ecosystems.

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3.26.P-Th117 Transformations of ZnO ENPs Change Everything: Bioaccumulation, Plant Health, and Soil Microbiome Response

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Engineered nanoparticles (ENPs) released into the environment, including soil, undergo physical, chemical, and biological transformations that alter their properties, mobility, and bioavailability in the soil-plant system. Pristine (p-ENPs) and transformed (trans-ENPs) ENPs differ in solubility, sorption, and bioaccumulation potential, which may promote plant growth or cause toxicity at higher concentrations. Significant knowledge gaps remain regarding the accumulation of Zn released from ZnO ENPs in edible plants. Furthermore, the impact of multiply transformed forms of ZnO ENPs on Zn bioavailability in soil and its subsequent transfer through the food chain remains unknown. The aim of this study was to determine the effect of ZnO ENP transformation on Zn accumulation in lettuce and spinach and to assess plant health indicators (chlorophyll, MDA level), with particular emphasis on potential risks to consumers. The next step was to determine the effect of ZnO ENPs on the activity of extracellular enzymes in soil and to determine the copy numbers of bacterial genes involved in C, N, and P cycling. Plants were grown in LUFA 2.3 soil enriched with p- or trans-ZnO ENPs or ZnSO₄ (20 and 200 mg Zn/kg). Chemical modification of ZnO ENPs involved sulfidation of ENPs (sulf-ZnO ENPs) by aging in sulfide ion solutions. Biological modification of ENPs consisted of protein-coated BSA (BSA@ZnO ENPs). Doubly converted ZnO ENPs were obtained by forming a protein corona on the sulfided ENPs (BSA@sulf-ZnO ENPs). The results show that Zn bioavailability in soil depends on the dose and form of ZnO ENPs, and accumulation in plants also depends on the species. The highest Zn concentrations in roots were observed after p-ZnO, and the lowest after ZnSO₄. Furthermore, p-ZnO demonstrated limited translocation, while ZnSO₄ demonstrated greater mobility. All risk indices (EDI, HRI, THQ) were <1, indicating no health

risk. Chlorophyll and MDA content depended primarily on the type of ENPs. Analysis of soil enzyme activity indicates that microorganisms adapt to ZnO ENPs, and longer exposure may even increase the activity of organic matter-degrading enzymes. It is worth emphasizing that trans-ZnO ENPs exhibit a different pattern of accumulation in soil and exert a different effect on plants and soil microbiota than p-ZnO ENPs. These findings represent an important step towards understanding the fate and effects of trans-ZnO ENPs in the soil-plant system.

Disclaimer/Disclosure:

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3.26.P-Th118 Potential risks of enhanced weathering for climate, human health, and ecosystems

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Enhanced weathering has recently gained attention as a promising CO₂ removal (CDR) practice, but previous analyses are based on theoretical assumptions that differ substantially from experimental observations and overlook the risks of heavy metal emissions. Here, we draw on the results of existing experimental studies to conduct a prospective life cycle assessment of alternative enhanced weathering scenarios. We find that initial CDR is insufficient to counterbalance greenhouse gas emissions occurring throughout the rock supply chains in some scenarios, only achieving net removal one to four decades after rock particles are applied on inland areas. Moreover, our results indicate that enhanced weathering could entail substantial damage to human health and ecosystems. Initially, these impacts are mainly linked to the rock transportation and quarry operations, but in subsequent years health risks could arise due to the potential release of toxic heavy metals. Contrary to the prevailing assumption, we find that zinc emissions from basalt could pose a greater health threat than nickel release from dunite. These preliminary results highlight the need for additional research—prioritizing non-agricultural areas to avoid food chain contamination—to demonstrate the short-term net climate benefits of enhanced weathering before CDR credits are commercialized.

Disclaimer/Disclosure:

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3.26.P-Th119 Arsenic bioaccumulation in alder trees (*Alnus* spp) from gold mine tailings, Nova Scotia, Canada: Implications for phytoremediation and environmental risk assessment

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Speckled alder (*Alnus incana* subsp. *rugosa*) is a subspecies of *Alnus incana* with a wide range across the cooler regions of Northern America. Speckled alder provides essential habitat for a variety of wildlife and serves as a food source for small mammals and birds. Speckled alder commonly becomes established at mining and industrial sites however little is known about its phytoremediation potential or the metal bioaccumulation risk it may pose. We examine concentrations of arsenic (and other metalloids) in speckled alders that have become naturally established at a legacy gold mine tailings impoundment in Nova Scotia, Canada. Field sampling involved collecting alder leaves, catkins, and branches, as well as associated tailings soil, and surface and groundwater samples, which were analyzed for metals using X-ray fluorescence (XRF) and inductively coupled plasma spectrometry (ICP-MS). Arsenic concentrations in tailings impoundment soil (100- 3200 ppm), surface water (352 -3440 ppb) and groundwater (4320 - 5620 ppb) exceeded Canadian environmental guidelines (12 ppm soil, 5 ppb water). Average arsenic concentrations were 1362 ppb in catkins, 914 ppb in leaves and 313 ppb in wood. Our data suggests that speckled alder growing on legacy gold mine tailings tolerates high concentrations of arsenic and, as it is endemic to the area, may be an effective phytoremediator. However, preliminary data from alder growing on nearby but undisturbed soil indicates much lower As levels suggesting the increased potential for As in speckled alder growing on mine tailings to bioaccumulate in local wildlife. Further sampling is underway to provide guidance on determining bioconcentration factors to more accurately assess both phytoremediation potential and environmental risk to biota in the region.

3.26.P-Th120 Industrial chemicals in irrigated agricultural soils: a case study on the Great Bačka Canal, Vojvodina Province, Serbia

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Industrial chemicals, together with other micropollutants, are increasingly found in agricultural soils, mainly due to irrigation with surface waters receiving wastewater discharges, as well as the use of treated wastewater or sludge in crop production. In Serbia's intensively cultivated Vojvodina Province, irrigation practices often rely on water from the Danube-Tisa-Danube Hydro-System. Within this canal network, the Great Bačka Canal represents one of the most contaminated stretches due to a prolonged history of industrial and municipal effluents. Continuous use of the Great Bačka Canal water for agricultural irrigation creates conditions in which soils may act as long-term repositories for a broad spectrum of different contaminants. Thus, the objective of this study is to characterise the occurrence and distribution patterns of industrial chemicals in soils exposed to irrigation with water from the Great Bačka Canal.

To assess this potential occurrence, 100 soil samples were collected from four agricultural fields regularly irrigated with water from Great Bačka Canal. The samples were processed using the QuEChERS extraction protocol and analysed by liquid chromatography - high-resolution mass spectrometry. A comprehensive suspect-screening workflow was implemented to identify industrial chemicals repeatedly occurring in the soil samples. Contaminants were ranked according to detection frequency and annotation confidence, after which selected candidates were quantified using reference standards. Among detected compounds were triphenyl phosphate, tributyl phosphate, dodecyl sulfate, 2,5-di-tert-butylhydroquinone, triethyleneglycol bis(2-ethylhexanoate), dibutyl phosphate, triethyleneglycol bis(2-ethylhexanoate), and triphenylphosphine oxide with maximum concentrations up to 300 ng/g. The results highlight the importance of robust monitoring schemes that capture realistic exposure scenarios in agricultural areas affected by contaminated water sources, thereby supporting more reliable ecological and human health risk evaluations.

Disclaimer/Disclosure:

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3.26.P-Th121 Pesticide Profiles in Irrigated Agricultural Soils from the Northern Serbian Province of Vojvodina

Jelena Živančev, Igor Antić, Maja Buljovčić, Dušan Rakić and Nataša Đurišić-Mladenović, University of Novi Sad, Faculty of Technology, Serbia

The presence of pesticide residues in agricultural soils reflects not only their field application but also the quality of water used for irrigation, which may serve as an additional pathway of contamination. In the northern Serbian Province of Vojvodina, farmers frequently rely on surface-water irrigation, and long-term use of water from impacted waterways can gradually alter the chemical status of soils. One such water source is the Great Bačka Canal (GBC), a section of the Danube-Tisa-Danube network historically burdened by diverse pollutant inputs. While its water quality has been studied extensively, much less is known about how irrigation with GBC water influences the pesticide residue patterns in surrounding soils.

To address this gap, this study investigated the occurrence of pesticide residues in soils collected from four agricultural sites that regularly rely on GBC irrigation. A total of 100 samples were analysed. Soil extracts were prepared using a QuEChERS-based procedure and analysed by liquid chromatography-high-resolution mass spectrometry. The analytical strategy combined broad suspect screening with targeted quantification of confirmed pesticide candidates.

Multiple pesticides were detected, including carbaryl, pyrimethanil, fluopyram, bentazone, metalaxyl, fluxapyroxad, metamitron, chloridazon, prometon, metolachlor and azoxystrobin, with concentrations reaching 201 ng/g. The distribution of residues points to the dual influence of agricultural use and long-term exposure to canal water. These findings emphasise the need to consider irrigation water quality when assessing pesticide accumulation in soils, particularly in regions dependent on surface water irrigation.

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3.26.P-Th122 Remediation of Pesticides by Vegetation Strips: the Uptake and Impact of Azoxystrobin, Diflufenzuron and 2,4-Dichlorophenoxyacetic Acid on the Species *Arabidopsis thaliana*.

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England is experiencing a river water quality crisis, with the Environment Agency (EA) classifying 85% of rivers as failing to achieve good ecological status in 2022. Pesticide contamination from agricultural pollution is a key contributor to impaired streams and is harmful to ecosystem health. This project aims to understand plant pesticide interactions, including uptake, and their impacts on plant growth and health. Additionally, we investigated pesticide adsorption rates to soils of varied composition to assess environmental bioavailability. Three pesticides were selected based on EA analysis of English rivers, prioritising pesticides by concentration and ecotoxicological risk. Initial experiments examined the uptake and plant response to azoxystrobin, diflufenzuron, and 2,4-Dichlorophenoxyacetic acid (2,4-D) by the model plant *Arabidopsis thaliana*. Pesticide exposures were conducted at three concentrations, 2.5, 5, and 10 µg/l, in controlled tissue culture experiments in ½ strength Murashige and Skoog Medium (0.75% agar) to investigate pesticide degradation rates, plant uptake, and plant stress responses. Each individual pesticide exposure had 6-8 replicate vessels, with 2 plants per 150ml of media, grown with 12:12 day: night light at 25°C and 70% relative humidity. Following 6 weeks of growth, plant stress was measured using ImageJ to determine plant leaf surface area and chlorophyll fluorescence to measure chlorophyll death. A pesticide mass balance was calculated using LC-MS measurements of pesticide concentrations in the media, root, and shoot biomass. Additionally, pesticide uptake, degradation, and diffusion rates in the media were calculated. Utilising the largely annotated *A. thaliana* genome, global proteomic and metagenomic analyses were carried out to determine plant pesticide response and the up and down regulation of plant stress response systems. Further experiments investigated pesticide bioavailability in varied soil types using ratios of organic matter to clay with partitioning experiments at 2.5, 5 and 10 µg/l. Solid phase extractions and LC-MS analysis were used to quantify the pesticide soil adsorption, and bioavailability. Vegetation strips are a valuable protection strategy for river water quality against agricultural pesticide pollution. With further understanding of plant pesticide uptake and their impact on plant health at a metabolic level, vegetation strips can be better designed to improve plant uptake.

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3.26.P-Th123 Tracking Environmental Organic Micropollutants in Soils and Traditional Catalan Calçots (green onions)

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Agricultural production is increasingly affected by global stressors such as population growth and climate change. Although several strategies have emerged, as the use of reclaimed water or the composting of WWTP's sludge, both may introduce OMPs into agricultural soils. This study investigates the presence of pharmaceuticals, antibiotics, plastic additives and other OMPs in calçots, a traditional winter onion grown in Catalonia, from diverse agricultural settings, including fields irrigated with groundwater, tap water, reclaimed water and those amended with composted sludge. Samples were collected during the 2024–2025 harvest, separated into roots, bulbs and leaves, freeze-dried and extracted by bead beating with acetonitrile. Targeted UHPLC-QTRAP analysis was conducted for 9 samples of soils and calçots from different farms from Catalonia. The target analysis enabled the detection of 109 compounds across the four matrices. Soils exhibited the highest diversity of contaminants, with recurrent detection of caffeine (8 samples), tolyltriazole (7), BPA (6), 6PPD-q (6), TCEP (4) and various pharmaceuticals. Roots showed fewer detections overall but consistent presence of TCEP and DPG in all samples, caffeine in 7, and β-blockers such as nadolol and propranolol in 6. Bulbs displayed an intermediate pattern dominated by methylparaben (8 detections), DPG (6), BPA (4) and tolyltriazole (4). Leaves showed frequent detections of TCEP (9), DPG (7), benzophenone-3 (7), caffeine (6) and occasional TBEP and ibuprofen metabolites. These patterns aligned with accumulation factors: several compounds exhibited mean ratios >1 in at least one comparison, including TCEP, BPA, DPG and caffeine for root/soil and bulb/soil; and caffeine, DPG and benzophenone-3 for leaf/soil. Conversely, many pharmaceuticals (e.g., azithromycin, trimethoprim, diclofenac) remained largely confined to soils, with ratios consistently <1. Overall, the results indicate that while most contaminants remain predominantly in soil, a subset of environmentally relevant OMPs—including TCEP, DPG, caffeine, BPA and benzophenone-3—can be detected

across plant tissues and may accumulate to higher levels than in soil. Ongoing wide-scope screening and a community survey to understand citizen perspective on micropollutants will further refine the interpretation of contaminant sources and help contextualize their relevance within current calçot production practices.

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3.26.P-Th124 Fate of Tire Wear-Derived Contaminants in Tire Wear Particles-Soil-Plant System

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Tire wear particles (TWP) are continuously introduced into soils through atmospheric deposition, road runoff, and biosolid application. TWPs consist of polymers, carbon black, and numerous additives, referred to as 'tire wear-derived contaminants' (TWCs). Once in the environment, TWCs can be released from the TWPs into soil, taken up by crops, and pose health concerns to ecosystems. Despite increasing evidence of TWCs in soils and plants, their fate in the environment, specifically, their release from TWPs and subsequent uptake by plants and metabolism, remains insufficiently understood. Current studies on plant uptake of TWCs were conducted under hydroponic conditions and/or using elevated TWCs concentrations and therefore do not reflect environmentally relevant cases. To address these gaps, this study examined the fate of six representative TWCs: benzothiazole (BTZ), 2-aminobenzothiazole (A-BTZ), 1,3-diphenylguanidine (DPG), hexa(methoxymethyl)melamine (HMMM), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD), and 6PPD-quinone, as well as their transformation product in soils mixed with TWPs (0, 0.1, 1, and 2.5%) serving as the source of TWCs for plant uptake.

Our data show that both alfalfa and lettuce were contaminated with TWCs originating from TWPs. Among the detected TWCs, DPG exhibited the highest concentrations in alfalfa and lettuce (168 and 454 ng/g, respectively), and the greatest number of transformation products (14 out of 21). BTZ and its transformation products were not detected in plant tissues, likely due to rapid in-plant metabolism. TWPs' level in soil affected the overall TWCs taken up; for example, DPG's concentration increased from 41 ng/g (0.1% TWPs) to 168 ng/g (2.5% TWPs) in the 1st alfalfa harvest. Additionally, TWCs concentration in the alfalfa shoot decreased over the growing season during the alfalfa growth period, which was harvested five times at the shoot base. For example, the concentration of A-BTZ in alfalfa grown in soil amended with 2.5% TWPs decreased from 33 ng/g in the 1st harvest to 4 ng/g in the 5th harvest. This decline is consistent with the rapid release of TWCs from freshly added TWPs and is further supported by the sequential water-extraction data. This study demonstrates that TWPs are a major source of tire wear chemicals in soils. Recognizing this consequential exposure pathway is vital for regulatory decision-making, environmental risk assessment, and food-safety protection.

3.27.A Strategies for PFAS, Organic Micropollutants & Their Persistent Products: Analysis, Assessment & Treatment Across Environmental Media

3.27.A.T-01 Understanding PFAS Exposure: Wide-scope Suspect Screening Reanalysis of High-resolution Mass Spectrometry Data from Exposomics Studies

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Per- and polyfluoroalkyl substances (PFAS) encompass thousands of structurally diverse anthropogenic compounds varying in fluorination, chain length, and functional groups. Their extreme stability and widespread use lead to a constant human exposure, yet poorly understood. Targeted studies focus mainly on about 30 regulated PFAS, capturing only a minimal fraction of the true PFAS exposome. Advances in high-resolution mass spectrometry (HRMS) have enabled non-targeted analysis (NTA) workflows, which exploit accurate mass, fragmentation patterns, and neutral losses to broaden the coverage of PFAS chemical space. However, NTA data processing remain biased toward common structural motifs and homologue series, uncovering atypical PFAS such as fluorotelomers or ethers. Plus, many exposomics studies focus narrowly on regulated PFAS, neglecting the broader spectrum of relevant homologs that may occur in study samples.

Retrospective analysis (reanalysis) of archived HRMS datasets using broad and chemically informed suspect lists has the potential to overcome these limitations. In this context, we propose a retrospective wide-scope suspect screening tool tailored for PFAS exposomics studies. The extensive suspect list was curated from experimental data on standard mixtures and the OECD PFAS inventory, filtered for compatibility with reversed-phase LC (> 1600 entries). Each suspect entry was paired with structural descriptors, predicted retention indices (RIs), and MS² fragments sourced from standards or, when unavailable, databases and literature data, also assigning relative identification confidence levels. Candidate features are prioritized by precursor accuracy, isotope, and MS² matching, while a curated filtering strategy based on spectral similarity, intensity, and identification confidence ranking minimized redundancies. PFAS identification accuracy was further refined by a method-dependent retention time-RI regression model.

Reanalysis by wide-scope suspect screening of LC-HRMS data on human serum from general and PFAS-exposed cohorts confirmed not only legacy perfluoroalkyl acids (e.g., PFOS, PFHxS, and PFNA) but also a wider list of unregulated PFAS isomers previously neglected—including fluorotelomer ethers, alcohols and acetates, unsaturated PFAS, sulfonamidoacetic acids, and amines—thereby broadening prior exposure assessments (about 6.5-fold increase compared to former identifications) and the knowledge on PFAS chemical coverage in exposomics.

3.27.A.T-02 Monitoring of PFAS in Urban and Industrial Wastewater via Sum Parameter and Target Analysis: The Critical Role of Particulate Load

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Since the initial reports indicating the potential of sum parameters of adsorbable organic fluorine (AOF), extractable organic fluorine (EOF), and total fluorine (TF) to detect and survey large quantities of unidentified organofluorine compounds in environmental matrices, their utilization has become a valuable addition to conventional PFAS analytical methods. The sum parameter AOF was the first parameter to become standardized through the DIN EN ISO 18127:2024-08-Entwurf standard. The EU-Wastewater Treatment Directive (EU-UWWTD) demands the implementation of a monitoring program for per- and polyfluoroalkyl substances (PFAS) in wastewater nationwide by 2027, following its enactment in late 2024. To date, only a limited number of studies have demonstrated the feasibility of sum parameter analysis in wastewater samples.

Herein, we investigated several wastewater treatment plants (influent and effluent) from urban areas as well as selected PFAS manufacturing industries. All wastewater samples were sampled via 24-h sampling auto-samplers or via qualified random sampling and subsequently worked, based on state-of-the-art extraction and measurement protocols. In order to obtain further information regarding the distribution of analytes in the wastewater matrix and to assess the applicability of the standard methods for "PFAS Total" and "Sum of PFAS", we applied target analysis by Liquid Chromatography Coupled Tandem Mass Spectrometry (LC-MS/MS) and AOF sum parameter analysis by Combustion Ion Chromatography (CIC). The evaluation of the data indicates the presence of significant trends, which can contribute to the selection of appropriate harmonized methods and strategies for future PFAS monitoring in WWTPs.

Overall, we demonstrated that a majority of PFAS in WWTP streams gets discarded in sludge sedimentation. However, highly soluble, short-chain compounds leave the treatment process untreated. Moreover, our study showed that sum parameter analysis combined with various extraction methods enables a thorough examination of PFAS-containing wastewater matrices and can be employed to evaluate PFAS levels in influent and effluent samples from urban and industrial wastewater streams. Since Trifluoroacetic acid (TFA), the major PFAS degradation product, was quantified in significant amounts for all samples investigated, an additional monitoring of WWTP effluents is highly recommended.

3.27.A.T-03 Long-Term Study of River Spree With Insights on Pollutions Situation With a Focus on PFAS

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Per- and polyfluorinated alkyl substances (PFAS) are environmental pollutants of concern regarding their persistency and bioaccumulation properties and impact on human health. Due to the wide range of existing physicochemical properties of PFAS molecules it is difficult to detect all compounds with one single method. In

Target Analysis missing analytical standards poses a problem. Additional measurement time and manual effort for data assessment create difficulties in Non-Target Screening. Within our study complementary sum parameter tools (extractable organic fluorine, EOF) were developed as a fast-screening tool for river water samples.

Through a long-term study of River Spree, we receive insights into the pollution situation regarding organic fluorinated compounds and organic trace substances. EOF measurements and subsequent complementary Target and Non-Target PFAS analysis give a more detailed insight into the environmental pollution situation with fluorinated compounds. Correlation assessment between fluorinated organic compounds and organic trace substances was conducted. The idea here was that, in the event of a correlation, it would be possible to infer exposure to PFAS from exposure to organic pollutants. Correlation tests showed increase of fluorinated compounds are coming with an increase of organic trace substances. However, the influence of season and weather (warm, dry, wet) leads to inconsistency of the significance of the correlation. Therefore, this study provides EOF as a Hot-Spot Screening Tool, shows the importance for improvement of analytical methods for PFAS detection and underlines the importance of long-term environmental studies as the time of sampling has an impact on their concentration.

Disclaimer/Disclosure:

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3.27.A.T-04 Mineralization of Per- and Polyfluoroalkyl Substances at a Municipal Solid Waste Incinerator

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Per- and polyfluoroalkyl substances (PFAS) in municipal waste poses challenges for safe disposal and circular economy objectives. To address methodological limitations in previous studies, a PFAS measurement campaign was conducted at a municipal solid waste incineration (Gemeinschaftskraftwerk Schweinfurt, GKS). The aim of the campaign was to investigate the thermal decomposition of PFAS in the grate furnace and the efficiency of the flue gas cleaning system. Three test scenarios were established: municipal solid waste (MSW) as the reference scenario, MSW mixed with PFAS-contaminated soil, and MSW with manually added PFAS-containing materials as a worst-case scenario. Input and output streams were sampled and analyzed. Regarding the gaseous emissions, U.S. EPA methods OTM-45 and OTM-50, variations of the OTM-45 and fourier-transform infrared spectroscopy (FTIR) and tunable diode laser (TDL) measurements for volatile PFAS and hydrogen fluoride (HF) were carried out.

Analyses of the MSW indicated PFAS concentrations of 40–50 µg/kg (sum of 43 substances), approximately half of it attributed to trifluoroacetic acid (TFA). The primary air contained 2–3 ng/m³ of 35 ionic PFAS, while process water contained 2 µg/L TFA and 6 ng/L other ionic PFAS.

The flue gas measurements showed total polar and semi-volatile PFAS concentrations of 0–22 ng/m³. Respectively no significant increase was caused through PFAS addition. It is likely that TFA emissions in the flue gas originated from pre-contaminated scrubber water rather than incomplete combustion. Carbon tetrafluoride (CF₄) emissions (FTIR: 1–2 mg/m³, OTM-50: 0.5 mg/m³) were observed only during PFAS addition, while other products of incomplete combustion were undetectable. Concentrations of PFAS in bottom ash residues were below 5 µg/kg.

3.27.B Strategies for PFAS, Organic Micropollutants & Their Persistent Products: Analysis, Assessment & Treatment Across Environmental Media

3.27.B.T-01 Biotransformation of Eight PFAS Classes in Three Fungal Cultures and Sewage Sludge – Impact on Primary Degradation and Transformation Product Formation

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Per- and polyfluoroalkyl substances (PFAS) are a class of over 4,700 synthetic chemicals, many of which are either highly persistent or can form persistent transformation products, contributing to global environmental contamination. While regulatory monitoring focuses on legacy compounds, thousands of per- and

polyfluoroalkyl substances remain understudied, with PFAS sum parameters indicating large amounts of unidentified organic fluorine.

Fungi are abundant in soils and wastewater systems and possess unique enzymatic capabilities. Yet, their role in per- and polyfluoroalkyl substance transformation remains poorly explored.

This study investigated the biotransformation of nine structurally diverse per- and polyfluoroalkyl substances belonging to eight substance classes in three fungal cultures, *Pleurotus ostreatus*, *Gloeophyllum trabeum*, and *Phoma* sp. UHH 5-1-03 as well as in activated sludge. The studied PFAS classes included perfluoroether carboxylic acids, a perfluorinated phosphinic acid, a fluorotelomer carboxylic and sulfonic acid, a side-chain fluorinated aromatic compound along with two sulfonamide derivatives. The analytes were spiked into the cultures as a mixture and the time points were sampled sacrificially. High-resolution mass spectrometry was used to track parent compound depletion and transformation product formation.

Five of the nine compounds showed no biotransformation across tested cultures, indicating limited biodegradation potential for many modern PFAS classes. Rapid biotransformation of 6:2 fluorotelomer carboxylic acid was observed in all cultures with half-lives below 20 hours. 6:2 fluorotelomer carboxylic acid also displayed a culture-specific formation of transformation products. The side-chain fluorinated aromatic compound was also biotransformed quickly. Notably, only *Pleurotus ostreatus* transformed perfluorobutane sulfonamide forming alcohol- and carboxylic acid conjugated metabolites that resemble phase II transformation products observed in mammals.

The results demonstrate that fungal species differ in transformation capacity and pathway selection leading to distinct transformation product profiles.

Disclaimer/Disclosure:

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3.27.B.T-02 Sewage sludge derived carbons as circular sorbents for the removal of persistent and mobile organic compounds

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Persistent and mobile organic compounds (PMOC) such as pharmaceuticals, personal care products, PFAS, industrial chemicals and their transformation products escape conventional wastewater treatment, challenging the new EU Urban Wastewater Treatment Directive. We tested whether wet wastes such as sewage sludge, after phosphorus (P) recovery via hydrothermal carbonization (HTC), can be converted into efficient sorbents by steam activation for advanced treatment. Hydrochars were produced from sewage sludge with or without lignocellulosic (bark, foliage, green waste) or nutrient-rich (manure) co-substrates, then steam-activated under varying temperatures and durations, including Fe-enriched and pyrolyzed controls. Sorbents were comprehensively characterized, and batch adsorption experiments were performed with real tertiary effluent from a decentralized wastewater treatment pilot (aerated constructed wetland, sand filter, UV) containing >200 PMOC (including transformation products) quantified by LC-HRMS. Molecular descriptors were computed (RDKit, ChemAxon) and linked to removal using correlation analysis, Logistic Regression and machine-learning models (Random Forest, XGBoost).

Steam-activated hydrochars from sewage sludge+bark removed up to twice as many PMOC as the corresponding biochar, driven by ~5-fold higher surface area, higher microporosity and basic mineral surface functionalities that support π - π , hydrophobic and H-bond interactions. Moderate activation temperature (800 °C) combined with longer duration maximized microporosity and sorption, whereas higher temperature (900°C), manure co-substrates and Fe-enrichment all reduced removal due to ash-driven pore blockage. Transformation products such as gabapentin-lactam and guanylurea were more strongly retained than their parents, consistent with reduced polarity.

Across >200 PMOC, hydrophobicity descriptors correlated positively with removal, while polarity descriptors correlated negatively. Ionization descriptors showed that neutral or protonated species sorbed best.

Classification models consistently identified lipophilicity (at pH 7), apparent aqueous solubility (at pH 7), aromaticity, polarizability, pKa(s) and solvent accessible surface area of PMOC as top features affecting sorption. HTC of sewage sludge, coupled with steam activation of the hydrochar, provides a promising pathway to recover P while producing a circular sorbent capable of meeting future EU micropollutant removal targets.

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3.27.B.T-03 Browsing FAIR Transformation Product Information with FAIR-TPs

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From identifying transformation products (TPs) in samples using mass spectrometry (MS) to incorporating them into chemical regulation, scientists still face significant challenges integrating TPs into environmental research. Yet, sharing information on TPs is more important than ever in the AI era. The FAIR-TPs website, presented here, lets users examine publicly accessible TP data gathered from various literature sources. The underlying data comes from several datasets on the NORMAN Suspect List Exchange (NORMAN-SLE) as well as ChEMBL, all accessible via PubChem. An open source workflow is used to compile the data, which are archived and versioned on Zenodo under a CC-BY license, and then processed and visualized on FAIR-TPs. At present, FAIR-TPs contains 11,082 reactions, involving 9,404 compounds from 11 sources.

The FAIR-TPs website is designed to make transformation knowledge reusable, testable, and accessible, providing features to investigate and produce outputs suitable for scientific dissemination. Because compounds and their reactions are highly interconnected, the data are stored in a graph-based structure and visualized through an interactive interface with statistics and several search options. The transformation data come from the “Transformations in PubChem – Full Dataset” hosted on Zenodo, including compound-level information (CID, InChI, InChIKey, SMILES, molecular formula, exact mass, xlogP, names/synonyms) and reactions-level data (biosystem, enzyme, transformation, massDiff, xlogpDiff and reference metadata).

During a chemistry-aware Transform step, identifiers are homogenized, names are normalized with a majority-vote method and long or problematic names are trimmed. In the Load step, the data are stored in a Neo4j graph compounds as nodes and each reaction as a directed edge, allowing efficient navigation of multi-step reaction networks. A Django interface renders structures, performs depth-limited traversals, and provides CSV/XLSX/patRoon/MetFrag downloads for MS workflows, plus image downloads. After statistics are verified for completeness, the site is deployed on a virtual machine, available at <https://fairtps.lcsb.uni.lu>. FAIR-TPs helps researchers connect precursors, intermediates and end products by facilitating the browsing, comparison, and reuse of transformation knowledge. This improves the basis for analysis, assessment, and treatment across environmental media, where understanding reaction pathways is essential.

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3.27.P Strategies for PFAS, Organic Micropollutants & Their Persistent Products: Analysis, Assessment & Treatment Across Environmental Media

3.27.P-Mo235 Destruction of Per- and Polyfluoroalkyl Substances Containing Emerging Aqueous Film-Forming Foam in a Full-Scale Hazardous Waste Incinerator

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Per- and polyfluoroalkyl substances (PFAS) are among the environmental contaminant groups that have received the most attention in recent years. The heightened concern arises from the fact that their unique molecular peculiarity responsible for widespread use, including in critical applications, simultaneously account for their environmental persistence.

The safe destruction of PFAS in various matrices, such as waste streams, is of great importance to prevent the impact on the environment and human health. The volume of such waste increased following the phase-out regulations of PFAS containing aqueous film-forming foams (AFFF) in numerous countries. Originally intended for firefighting, they are now classified as hazardous/ toxic waste that must be properly destroyed.

Incineration shows high potential for destruction of PFAS in impacted wastes since it can break the strong carbon-fluorine bond. This could potentially lead to completely mineralization of PFAS. However, previous experience with halogenated waste incineration has shown that products of incomplete destruction (PIDs) can be formed if the incineration conditions are insufficient.

In the project „Investigating the Thermal Decomposition of PFAS in a Full-Scale Commercial Hazardous Waste Incinerator” funded by the U.S. Department of Defense’s Strategic Environmental Research and Development Program (SERDP), German, Australian and American partners examine the treatment of PFAS containing waste streams in a full-scale hazardous waste incineration plant to demonstrate the readiness, viability, and level of safety of this destruction method.

Here, we present the results of our first campaign. We focused on the incineration of a contemporary fluorotelomer-based AFFF under varying conditions to evaluate the completeness of PFAS destruction and the potential formation of PIDs. In addition to solid samples (slag, boiler ash and dust) and liquid samples (slag cooling water and scrubber water), flue gas was sampled and analysed.

3.27.P-Mo236 Analysis of per- and polyfluoroalkyl substances (PFAS) in atmospheric deposition

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The monitoring of per- and polyfluoroalkyl substances (PFAS) in the environment is of great interest due to their toxicity and persistence. Recent research has predominantly focused on measuring PFAS in soil and water, while studies analyzing PFAS in air—such as through stack emissions, ambient air, and atmospheric depositions—remain limited. This gap is partly due to the wide variety of physico-chemical properties among PFAS compounds and the need for diverse sampling techniques and analytical approaches. This study aims to evaluate the efficiency of a sampling technique for the atmospheric deposition of various PFAS, including polar, non-polar, volatile, and semi-volatile compounds. Tests were conducted in both real and controlled environments to assess the suitability of the method for PFAS with carbon chains ranging from C1 to C18. In addition, this work compares different analytical approaches for sample preparation and analysis as conducted by three independent laboratories.

3.27.P-Mo237 Advanced Ozone and Activated Carbon Operational Strategies for Enhanced Micropollutant Removal from Secondary Wastewater Effluent

Zoë Mol, Tao Zhang, Kristof Demeestere and Stijn Van Hulle, Ghent University, Belgium

Water resources are threatened given the fact that (emerging) contaminants are continuously introduced into water bodies. In view of providing safe water to humans and our aquatic ecosystems, current policy and legislative frameworks are stressing the need for advanced yet cost-effective water treatment approaches. Advanced oxidation processes are key in this context, but their performance is often limited by interactions between the organic matrix components (e.g. humic acids), the target micropollutants (μPs) and the added or generated oxidants. Ozonation is one of the most performant technologies, yet its dosing must be carefully optimized to avoid excessive energy use and undesirable by-products such as bromate. Combining ozonation with activated carbon (AC) adsorption can enhance μP removal while mitigating these drawbacks, though determining the optimal dosing control and sequencing of both processes remain challenging.

This study investigates various ozone and powdered AC (PAC) dosing strategies, with a focus on their sequential order. When applying ozonation first, or when combined with simultaneous AC addition, the μP removal efficiency increases by 5–10 % and the time to achieve 80 % removal halves, compared to the situation where AC is dosed prior to ozone addition. Pre-ozonation modifies the properties of effluent organic matter (EfOM) (e.g., hydrophobicity), reducing its competition with μPs for AC adsorption sites, while AC pre-adsorption shows limited benefit on the subsequent ozonation process. These findings support applying ozonation (optimal dose of 0.5 mg O₃/mg DOC) before or simultaneously with AC addition, followed by an AC polishing step, to achieve efficient μP removal while minimizing the formation of by-products.

Reliable real-time monitoring is essential for effective process control. Although an optimal ozonation dose of ~0.5 mg O₃/mg DOC is recommended, current practice lacks reliable strategies to balance O₃ and AC dosing in real time, often leading to overdosing, increased costs and carbon footprint, or insufficient μP removal.

Although surrogate parameters have shown promise in stand-alone ozonation or AC systems, integrated monitoring of the combined process is still scarce. This study therefore establishes a whole-process surrogate strategy for real-time optimization of O₃ and AC dosing, enabling more efficient control and supporting the broader implementation of intelligent tertiary treatment for micropollutant removal.

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3.27.P-Mo238 Comparing Oxidative Treatments of Triazole Fungicides and Their Persistent Transformation Products in Wastewater

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Degradation of micropollutants often results in the formation of intermediate transformation products (TPs). Many micropollutants are classified as persistent, mobile and toxic (PMT) or very persistent and very mobile (vPvM), reflecting limited degradation under natural environmental conditions. Despite this, they can still form TPs through, for example, oxidation processes used in advanced water treatment. These TPs may be more persistent, mobile, and toxic than the parent compounds making their formation and accumulation particularly concerning. Triazole fungicides are a group of micropollutants characterised by a 5-membered ring containing three nitrogen and two carbon atoms, which is responsible for their antifungal and toxic properties. During degradation of triazole fungicides, TPs typically retain the triazole ring or form 1,2,4-triazole as a standalone compound. Both photodegradation and biodegradation have limited effects on the 1,2,4-triazole ring. Although chemical oxidation processes may initiate reactions that promote ring opening and degradation of the 1,2,4-triazole ring, their ability to fully mineralise triazole fungicides remains unclear. Additionally, knowledge on the TPs formed during chemical oxidation processes, especially concerning the 1,2,4-triazole ring, is limited. In this study we aim to compare oxidative treatment of wastewater using ozone and heat-activated persulfate to provide insight into the ability of different radical species ($\text{OH}^\bullet$ and $\text{SO}_4^{\bullet-}$) to degrade and mineralise 1,2,4-triazole and its precursors. We will also investigate potential variations in degradation pathways between the two oxidation methods. TPs will be detected and measured via a suspect screening using high resolution mass spectrometry. The analysis will progress from simplified model water matrices to real and complex wastewater effluent matrices. We hypothesise that the two oxidation processes will produce distinct transformation pathways and TPs. We also expect that the increasing complexity of the water matrix will lead to a higher diversity of TPs. The findings will provide insight into the fate and persistence of triazole fungicides during chemical oxidation treatments.

3.27.P-Mo239 Mitigating the Ecotoxicity of Bentazone and Glyphosate using Combined Vacuum UV and UV-C Treatment of Contaminated Water

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Bentazone and glyphosate are examples of widely used herbicides that are frequently detected as contaminants in groundwater and surface waters. Bentazone (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) is a selective herbicide that inhibits photosynthesis in target plants, and glyphosate (N-(phosphonomethyl)glycine) is the most common active ingredient in non-selective broad-spectrum herbicides. The environmental toxicity of these herbicides to non-target organisms is considered moderate but adverse effects have been observed in aquatic organisms from different trophic levels including bacteria, algae, invertebrates, amphibians, and fish. Environmentally friendly methods for mitigation are therefore in demand. This study investigated the potential of combined vacuum UV (185 nm) and UV-C (254 nm) irradiation (VUV-UVC) as a mitigation method to degrade bentazone and glyphosate and thereby decrease their ecotoxicity. VUV treatment is an advanced oxidation process that degrades many aquatic contaminants, but the efficiency and the toxicological profile of the process is often underinvestigated. In this study, the UV mediated photolysis of bentazone and glyphosate was quantified by LC analysis, and the potential ecotoxicity of UV treated herbicides was examined using a battery of test organisms from different trophic levels including the yeast *Saccharomyces cerevisiae*, the microalga *Raphidocelis subcapitata*, the crustacean *Daphnia magna*, and selected annual broadleaf non-target plants. In addition, mesocosm with freshwater plankton was examined before and after exposure to UV treated herbicides. Different bioassays were included to better assess adverse effects of bioactive products in UV treated samples including herbicide transformation products. The study showed that combined VUV and UVC irradiation facilitated rapid photolysis within minutes of aqueous bentazone and glyphosate, and that the degradation was related to UV fluence (Joule/cm^2) and flow regimes. UV treatment of water contaminated with bentazone or glyphosate resulted in significant decreases in potential ecotoxicity due to complete mineralization or formation of less toxic transformation products. The results are relevant for assessing sustainable treatment of herbicide contaminated water with before discharge into environmental waters.

Disclaimer/Disclosure:

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3.27.P-Mo240 The Importance of Non-Targeted PFAS Analysis: Identifying Non-Regulated PFAS in UK Freshwater Samples by LC/QTOF

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Per- and polyfluoroalkyl substances (PFAS) represent a vast and diverse group of chemicals, yet most environmental monitoring programs rely on limited target lists, potentially leaving many PFAS undetected. This study demonstrates the value of non-targeted PFAS analysis through a case study of five surface water samples collected near a UK fluorochemical plant. These samples were analyzed using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC/QTOF MS), supported by FluoroMatch and MassHunter software for comprehensive annotation. Across the samples, 43 PFAS were identified at confidence level ≥3, spanning five structural classes, including perfluorocarboxylic acids (PFCAs), hydrogen- and chlorine-substituted PFCAs, perfluoroether carboxylic acids (PFECAs), and a suspected group of unsaturated PFECAs (UPFECAs). Notably, only eight of these compounds appear on the UK Drinking Water Inspectorate target list, representing just 17% of the total PFAS peak area detected. These findings highlight the limitations of targeted monitoring and underscore the importance of incorporating non-targeted screening to inform regulatory frameworks and ensure comprehensive environmental PFAS assessment.

3.27.P-Mo241 Fast Fluorine Screening Method for Sealing Materials via High Resolution-Continuum Source-Graphite Furnace Molecular Absorption Spectroscopy

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Elastomers commonly used as sealing materials are highly networked rubbery polymers. They are often produced using per- and polyfluoroalkyl substances (PFAS) either during manufacturing or as additives to enhance their performance during applications in harsh environments. Due to the lack of reliable recycling strategies without compromising the resulting polymer-quality, sealing materials are often disposed of in landfills. Thus, they represent a possible source of continuous PFAS contamination of both the immediate environment and surrounding ground water reservoirs through landfill leachates. To assess the scope of fluorine content, we developed a potential Standard Operating Protocol (SOP) to allow for subsequent fluorine/PFAS determination in various blends of sealing materials. Optimizations were aimed for time efficiency and robustness of results. The SOP entails a shredding sequence from roughly cutting to sieving. Optimal results were obtained for the fraction size between 125-250 µm. Extractions performed with either acidified methanol or ultra-pure water proved reproducibility of results and revealed high extractable fluorine concentrations for most sealing materials investigated. The high fluorine concentrations extracted by water further puts emphasis on the risks associated with leaching from microplastics generated by material abrasion during their lifetime and within landfills. The SOP can easily be expedited by implementation of additional sample preparation steps such as Solid Phase Extraction (SPE) to explicitly measure extractable organofluorides. In prospect of the broad ban of PFAS proposed by the European Chemical Agency (ECHA), it will be imperative to reliably screen products for fluorine and PFAS and scan for future compliance. Regarding the derogations discussed for certain applications such as Nafion proton exchange membranes and sealants in industrial sites, the SOP will allow for the fast screening of fluorine leaching to help advance the development of materials safe and sustainable by design.

3.27.P-Mo242 Metrology to support the analysis of per- and polyfluoroalkyl substances from industrial emissions (MetZeroPol)

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The EU’s Zero Pollution action plan aims at reducing pollution in air, water and soil to levels no longer considered harmful to health and natural ecosystems. As part of this, the Industrial Emissions Directive was

revised in 2024 which includes a magnitude reduction in the permissible associated emission level of a range of pollutants, affecting many industrial processes. Pollutants such as perfluoroalkyl and polyfluoroalkyl substances (PFAS) are increasingly causing concern. Up to now there is only little knowledge on which industrial processes emit PFAS, at what concentrations, and no European standard exists (only national standard in Belgium and France) for measuring PFAS emissions. Therefore, the Euramet project MetZeroPol will develop methods to measure PFAS and used to identify key PFAS species from real industrial processes. Furthermore, the members are involved in the new CEN/TC 264/WG 48 - “Emissions and ambient air - Determination of PFAS” group in order to uniformly regulate the standardization of analysis methods for PFAS emissions from industrial plants.

3.27.P-Mo243 Enhancing Per- and Polyfluoroalkyl Substances (PFAS) Pollution Retention by Superabsorbent Polymer (SAP) amended to a Grass Swale Prototype of Green Infrastructure

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Per- and polyfluoroalkyl substances (PFAS), or “forever chemicals”, are contaminants of escalating global concern. Superabsorbent polymers (SAPs), known for their high-water retention capacity, offer a potential new approach for enhancing PFAS removal within green stormwater infrastructure (GSI), like grass swales, particularly in the face of climate change-driven weather events in urban environments. Through the support of the Marie Skłodowska-Curie Actions European Union Programme, this project (PFAS-RETENT) will focus on evaluating the retention potential for a group of highly mobile, short-chain PFAS, using a biological (lignin)-based, superabsorbent polymer (SAP) integrated into a grass swale framework or prototype. To accomplish this, these specific objectives (OBJs) will be employed: OBJ1—evaluation of the retention-release processes of the SAP for the PFAS in focus (short-chain: perfluorobutanoic acid (PFBA), hexafluoropropylene oxide dimer acid (HFPO-DA), 4,8-dioxa-3H-perfluorononanoic acid (DONA), ultra short-chain: trifluoroacetic acid (TFA)), using batch retention experiments (simplified framework); OBJ2—assessing the efficacy of the SAP for removing these PFAS, when incorporated into lab-scaled, grass-planted microcosms (basic framework of a grass swale system); OBJ3—assessing the efficacy of the SAP in removing these PFAS when incorporated into flow-through, grass-planted mesocosms (‘real-world’ framework of a grass swale system). The methodology will follow a 3-stage stepwise approach, beginning with simple designs (batch experiments—OBJ1, microcosms—OBJ2), then increasing in complexity to a nearly real-world scenario (flow-through mesocosms—OBJ3). For OBJ2 and OBJ3 experiments, percolation and drought simulations will be applied in order to evaluate the SAP retention performance for PFAS under these conditions. SAP integrated into swale substrates could serve as a temporary “removal” barrier for short-chain PFAS during storm runoff, thereby limiting their distribution throughout urban water systems and the water cycle. During drought periods, PFAS trapped in the water-saturated gel phase of the SAP (along with the water) could be taken up by the vegetation within swale GSIs, thus reducing their release to nearby water bodies and minimizing contamination risks to urban water resources.

Disclaimer/Disclosure:

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3.27.P-Mo244 Quantification of 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 (PFAS), 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 liquid chromatography. For quantification of ultrashort-chain PFCAs (carbon chain length of two or three carbon atoms) often hydrophilic interaction liquid chromatography or ion chromatography coupled with mass spectrometry is used (e.g. DIN 38407-53). 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 two-digit ppt-range for all four analytes. The method was then tested on various real water samples, including tap and surface water, groundwater, landfill leachate, treated wastewater and impinger water from a sewage sludge incineration experiment. 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.

3.27.P-Mo245 Emerging Topics in the Monitoring of Organic Micropollutants in Surface Waters

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Driven by recent discoveries about the environmental impact of widely used organic chemicals, the EU has introduced new regulations for municipal wastewater treatment (UWWTD, 2024) and drinking water production (DIRECTIVE (EU) 2020/2184). Our team has responded flexibly by developing and innovating reliable analytical methods to detect emerging or previously undetectable organic micropollutants. The methods are suitable for routine analysis of various water types. Our presentation highlights four highly topical issues affecting water quality.

The revised Urban Wastewater Treatment Directive mandates the monitoring of 12 pharmaceutical and personal care products (PPCPs) commonly detected in wastewater. These substances are classified into two categories: easily treatable substances and easily disposable substances.

1,2,4-triazole is a persistent organic compound primarily found in the environment as a degradation product of various triazole fungicides commonly used in agriculture. It is also a key component in various pharmaceuticals, including antifungal, antiviral and antibacterial drugs.

Trifluoroacetic acid (TFA) has a wide variety of recognized anthropogenic sources in the environment. TFA arises from the degradation of previously used refrigerants and enters the environment through atmospheric deposition. Moreover, it is also a degradation product of long-chain PFAS. Regardless of its source, TFA ultimately accumulates in surface waters, reaching concentrations of units to tens of µg/l.

Chlorothalonil, a widely used fungicide that was banned in the EU in May 2020, had an estimated total consumption of 500 tonnes in the Czech Republic between 1997 and 2020. Initial results show that chlorothalonil 471811, a key metabolite, occurs year-round in surface waters of agriculture areas, reaching concentrations of several hundred ng/l. Removing this metabolite during drinking water production is challenging due to insufficient capture on granular activated carbon.

3.27.P-Mo246 Monitoring of Perfluorinated Compounds (PFAS) in Waters of the Vltava River Basin

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PFAS (Per- and polyfluoroalkyl substances) constitute a large group of synthetic chemicals (nearly 15 000, over 4700 with CAS numbers) with diverse physicochemical properties. Their high stability, resulting from the robust carbon-fluorine bond, combined with useful physicochemical properties has led to widespread use in various industrial and consumer products, including food and beverage packaging, clothing, cookware, cosmetics, paints and coatings, lubricants and firefighting foams. Nevertheless, these same properties now raise concerns about environmental pollution and human health. People are exposed to PFAS mainly through food and water, leading to bioaccumulation. The toxicity and health hazards associated with PFAS exhibit significant variability. Certain PFAS are suspected carcinogens and reproductive toxicants. The ability of modern analytical techniques to determine PFAS at trace concentrations has increased the importance of monitoring these persistent organic pollutants in the environment. Recent toxicological studies have led to a significant reduction in the acceptable daily intake levels for certain PFAS in human consumption. Consequently, both European and Czech legislation are being updated to reflect these new scientific findings.

The water management laboratories of the Vltava River Basin have been monitoring selected PFAS in surface and wastewater since 2023. A direct injection LC-MS/MS method for 20 PFAS, with detection limits of 0.5 to 6 ng/l, has been developed and validated to comply with Czech regulations. A parallel off-line SPE method using a Labtech XANA and D-EVA system was subsequently developed to achieve significantly lower detection limits required by stricter European directives.

The article presents and statistically analyzes the findings of perfluorinated compounds in waters within the

Vltava River basin in 2024. The obtained results most frequently showed the presence of perfluoroalkyl acids (PFOA, PFHxA, PFHpA and PFPeA).

3.27.P-Mo247 Development and Application of an Accurate Mass PFAS Library for GC/Q-TOF Analysis in Environmental Samples

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Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants detected in water, soil, and biota, posing significant analytical challenges due to their chemical diversity and volatility. We report the creation of an accurate mass Personal Compound Database and Library (PCDL) for PFAS analysis using high-resolution GC/Q-TOF instrumentation. The library comprises ~150 EI spectra with accurate m/z values, retention times, and structural identifiers, enabling both targeted and non-targeted workflows. Soil and drinking water samples were extracted using optimized solid-phase and solvent-based methods as well as SPME, followed by full-spectrum acquisition and screening. The PFAS PCDL facilitated confident identification of multiple PFAS classes, including fluorotelomer alcohols and perfluoroalkyl ethers, in environmental matrices. Additionally, complementary screening using NIST and pesticide libraries revealed co-occurrence of pesticides, PAHs, flame retardants, and industrial by-products. This approach demonstrates the utility of accurate mass libraries for comprehensive contaminant profiling and supports regulatory monitoring of emerging pollutants in complex environmental samples.

3.27.P-Mo248 Analyzing Ultra-short To Long Chain PFAS In Water Samples In A Single LC-MSMS Method With Maximized Sensitivity

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The analysis of per- and polyfluorinated alkyl substances (PFAS) in aquatic matrices has significantly evolved in recent years. The chemical properties of the analytes of interest are covering a very broad range, including highly polar analytes such as trifluoroacetic acid (TFA) to very non-polar analytes like perfluorooctadecanoic acid (PFOcDA). Classic reversed-phase LC columns with C18 stationary phases work very well for separating homologous rows of PFAS analytes from medium to low polarities and even their branched isomers, but highly polar analytes are not retained sufficiently and therefore cannot be separated properly. On the other hand, HILIC columns can be used to retain and separate highly polar PFAS analytes, while the non-polar long-chain PFAS remain unseparated.[1]

With recommended drinking water concentration limits are getting even lower, lately,[2] there is a need for increased sensitivity, allowing lower quantification and detection limits.

Here we show how a specially designed mixed-mode stationary phase LC column can be used to achieve good retention and separation across the full range of polarity of PFAS analytes. This allows the analysis of water samples from different types of sources, using a single chromatographic method.

Furthermore, we demonstrate maximized sensitivity in case of SPE sample preparation prior to analysis, as well as in a dilute-and-shoot application.

3.27.P-Mo249 Ecotoxicogenomic Hazard Assessment of the Sweeteners Saccharin and Cyclamate for *Daphnia magna* and Investigation of Their Biodegradability

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Artificial sweeteners such as saccharin and cyclamate are widely used as food additives because they provide high sweetness with low calorie intake. Both compounds are characterized by high water solubility and chemical stability, which contributes to their incomplete removal in wastewater treatment plants. Consequently, saccharin and cyclamate are frequently detected in treated effluents and surface waters at concentrations up to the microgram-per-liter range. Their persistence and mobility in aquatic environments raise concerns about potential ecological effects due to long-term environmental exposure. Although previous studies have indicated microbial involvement in their degradation, the identity of the responsible species remains insufficiently understood. The sweetener sucralose has previously been shown to alter the swimming behavior of *Daphnia*

magna, while comparable data for saccharin and cyclamate are largely lacking, and ecotoxicological information on these compounds remains scarce.

The aim of this project is twofold: First, to contribute to the hazard assessment of saccharin and cyclamate by addressing the data gap regarding their toxicological properties, using *Daphnia magna* as a model organism. Behavioral and acute immobilization tests were conducted across environmentally relevant and high exposure concentrations. First results showed that saccharin and cyclamate do not cause significant changes in swimming activity and lethality of *D. magna*. Extracted RNA was then sequenced to identify possible gene expression alterations caused by the sweetener.

Second, it seeks to identify microorganisms capable of degrading these sweeteners. This research will contribute to efforts aimed at reducing the abundance of saccharin and cyclamate in aquatic ecosystems. To investigate the degradability of saccharin and cyclamate, solutions containing each sweetener as the carbon source were inoculated with sewage sludge from a wastewater treatment plant. Actively growing cultures could be enriched for both sweeteners. In the following steps, these will be identified after enrichment by colony PCR and 16S sequencing.

This integrated approach, combining ecotoxicological hazard assessment with microbial degradation studies, will not only enhance the understanding of the environmental impact of saccharin and cyclamate but also support the development of effective bioremediation strategies.

3.27.P-Mo250 Elucidating Organohalogen Micropollutant Interactions with Clay Minerals: High Adsorption Indicates Relevance for Environmental Fate Processes

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Hydrophobic halogenated micropollutants widely show persistent, bioaccumulative, and toxic characteristics that lead to adverse effects on humans and other biota. The knowledge of sorption processes is highly relevant to estimate their mobility and retention and thereby to assess their environmental fate and risk. Their interactions with organic matter are intensively studied, whereas data on interactions with clay minerals (CM) are still scarce, especially for neutral, hydrophobic organohalogen compounds (OHC). To mechanistically elucidate OHC–CM interactions, we combined laboratory experiments with molecular modeling.

We studied OHC–CM interactions with five halogenated benzenes (log KOW 2.6–6.5) as well as twelve pure phyllosilicate CMs and 21 smectite-rich bentonites as mineral phases in laboratory adsorption experiments. Molecular modeling was performed at the density functional theory (DFT) level for the same five OHCs and montmorillonite models of varying mineral structure with and without explicit solvation.

Our results show that OHCs can adsorb to clay minerals to a relevant extent, with a strong mutual influence of OHC and CM properties on the extent of adsorption. Expectedly, the hydrophobicity of the OHCs played a crucial role for the extent of adsorption but can be outweighed by mineral properties. Results from molecular modeling did align well with laboratory results but partly deviated based on hydration state.

In particular, our results i) show the relevance of CMs for OHC adsorption with a wide variation of solid-liquid adsorption coefficients over orders of magnitude (log K_d <1.0 to >5.0); ii) provide mechanistic understanding of the influence of CM characteristics such as layer charge, exchangeable cations, particle size, and pore size distribution on adsorption; and iii) elucidate the hydration state to be critical both for the extent of adsorption energies and the type of interaction processes.

Overall, our results suggest a strong influence of specific OHC–CM interactions on the environmental distribution of OHCs and challenge the widely accepted paradigm that neutral (highly) hydrophobic compounds are (almost) exclusively sorbed to organic matter.

Thereby, our results highlight the relevance of minerals for the fate, transport, and retention of OHCs in the environment, e.g. for the elucidation of long-term source/sink phenomena in soils and sediments as well as for applications in water treatment and landfill sealing.

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3.27.P-Mo251 From Paper Food Contact Materials to Human Exposure: Characterizing PFAS Contamination, Migration Dynamics, and Health Risks

Paper-based food contact materials (FCMs) have been found to contain per- and polyfluoroalkyl substances (PFASs). Under high-temperature short-time (HTST) and long-term storage (LTS) conditions, PFASs from paper FCMs can migrate into dry and lipophilic foods. However, apart from using food simulants like 50% ethanol, there is a lack of research on the representativeness of other simulants, such as vegetable oil and Miglyol 812, for lipophilic foods. The objective of this study was to assess PFAS migration from paper FCMs to lipophilic foods under both HTST and LTS conditions. This study compiled occurrence data on per- and polyfluoroalkyl substances (PFASs) in FCMs from domestic and international sources collected between 2013 and 2024. The materials examined included paper bags, boxes, cups, plates, and grease-resistant paper, with a focus on perfluorocarboxylic acids (PFCAs, C4–C18), perfluorosulfonic acids (PFSAs, C4–C10), and PFAS precursors. To bridge regional monitoring gaps, 45 paper bag and box samples were collected from various retail channels in Taiwan. Samples were extracted using ultrasonic-assisted methanol extraction (60°C) and analyzed by LC–MS/MS and GC–MS/MS, following the CEN/TS 15968:2010 standard for targeted screening. Method validation confirmed recovery rates of 70–130% and detection limits of 0.5 µg/m². AKTS SML software was employed to simulate migration under HTST and LTS conditions. Migration simulations were conducted in accordance with EU and U.S. FDA regulations. The relative potency factor (RPF) approach was applied to convert individual PFAS exposures to PFOA equivalents for assessing human health risks. The results showed that precursors, specifically 8:2 fluorotelomer alcohol (8:2 FTOH), dominated in paper bags, significantly exceeding PFCAs, PFSAs, and other classes of compounds. Conversely, paper boxes showed markedly lower levels, indicating that paper bags were the primary source of concern. Under HTST conditions (175°C, 2 h), 50% ethanol proved to be the most effective extraction method, with precursor migration rates observed in paper bags. Crucially, LTS conditions (25°C, 15 months) resulted in 57% precursor migration, substantially surpassing standard simulation predictions (40°C, 10 days). This suggests current protocols may underestimate cumulative exposure risks. Exposure assessment confirmed HTST contact as a critical scenario, with 8:2 FTOH in paper bags presenting the highest exposure load.

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3.27.P-Mo252 Method Validation for the Analysis of per- and polyfluoroalkyl substances (PFAS) in Water Samples using Solid Phase Extraction and Liquid Chromatography coupled to Tandem Mass Spectrometry

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PFAS are a group of persistent chemicals widely used in various industrial and consumer products. Due to their persistence and potential adverse health effects, rigorous and sensitive analytical methods are needed that can monitor these compounds [1].

The Council Directive 2020/2184 of 16 December 2020 regarding the quality of water intended for human consumption states the parametric value for “Sum of 20 PFAS” of 0,1 µg/l and that the analytical methods should allow the quantification for “Sum of 20 PFAS “ at 30% of the parametric value [2].

The objective of this work was the implementation and validation of an analytical method for analysing the “Sum of 20 PFAS” in raw water and water for human consumption, namely: PFBA, PFPeA, PFBS, PFHxA, PFPS, PFHpA, PFHxS, PFHpS, PFOA, PFOS, PFNA, PFNS, PFDA, PFDS, PFUnDA, PFUnDS, PFDoDa, PFDoDS, PFTrDA and PFTrDS. The method was validated by applying several statistical tests: linearity, precision, accuracy, method quantification limit and matrix effects. Simultaneously, the prevention and mitigation of cross-contamination of these compounds in the laboratory was also studied.

Mass spectrometer parameters were optimized for each PFAS and two different MRM transitions were selected. Some compounds showed significant ion suppression effects (-57,5% for PFBA, -49,5 for PFPeA, -40,6% PFHxA, - 33,6% for PFHpA). For those compounds surrogate labelled compounds were used for results correction. This method showed excellent linearity ranges for all compounds (between 0,150 and 1,500 µg/L), with correlation coefficients greater than 0,9993. Recovery studies in several matrices with different fortification levels were performed using solid phase extraction with recoveries between 68% (PFDoDs) and 102% (PFOS) with RSD lower than 14,9% (PFBS). The Method Reporting Limit set for all compounds was 1,5 ng/L. The expanded uncertainty (k=2) of the analytical method was below 34,9% (PFBS). Interlaboratory studies showed

good performance for this method with z-scores of -0.5 for PFOS and -1.87 for PFOA.

Between 2023 and 2025 an extensive monitoring campaign was carried out in the EPAL and Águas do Vale do Tejo (AdVT) supply system to investigate the occurrence of PFAS in drinking water, surface water and groundwater. The compounds that were commonly found in drinking water and surface water were: PFBA, PFPeA, PFBS, PFHxA, PFHxS, PFOA and PFOS. None of the monitored PFAS were found in groundwater samples.

3.27.P-Mo253 Dissolved Organic Matter Accelerates the Aquatic Photochemistry of the Rubber Additive Diphenyl Guanidine (DPG)

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Diphenyl guanidine (DPG) is a widely used rubber vulcanizer that has emerged as an environmental contaminant of concern. While attention has focused on phenylenediamines like 6PPD and their toxic transformation products, DPG has been detected at relatively high concentrations in diverse environmental matrices. Despite its abundance, little is known about the environmental fate, transformation pathways, and toxicity of DPG. This study aims to address critical knowledge gaps by investigating the direct and indirect photochemical degradation pathways and kinetics of DPG in aqueous systems. Aqueous photochemistry experiments were performed using lamps centred at UV-B in solutions with and without dissolved organic matter (DOM) to explore the difference between direct and indirect photochemistry. DPG quantitation was performed with high-performance liquid chromatography with UV detection. We found that DOM from the Mississippi River increased the removal rate of DPG in a concentration dependent manner by up to 8x. Transformation products were identified using Orbitrap high resolution mass spectrometry. Phenylguanidine was formed in experiments both with and without DOM and confirmed with a standard. We tested the photochemical rate constant of phenylguanidine and found it was twice as fast as DPG. In direct photochemistry experiments, an oxygen addition transformation product [DPG+O] was identified. Ongoing work will explore the structure of this oxidation product; chromatographic peaks imply there are multiple sites of oxidation on structure. These findings have implications for understanding the environmental persistence and risks associated with DPG as a rubber-related chemical, as well as its transformation products, which are generally more water soluble and thus more mobile.

3.27.P-Mo254 Ecotoxicological Monitoring of the Photocatalytic Degradation of Micropollutants by Nano Metal-Oxide Based Photocatalysts

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Within the framework of the circular economy, it is essential to identify and develop technologies capable of treating wastewater generated by various industrial activities so that the treated and recovered water meets the quality requirements for agricultural reuse. However, current wastewater treatment processes often fail to remove numerous micropollutants, which—upon entering the environment—may harm ecosystems, disrupt biological functions, and bioaccumulate in organisms. Although several emerging processes can remove micropollutants, they may also generate metabolites or by-products with toxicity comparable to, or even exceeding, that of the parent compounds. Ecotoxicological assays therefore provide an excellent tool for monitoring treatment performance and evaluating whether degradation processes truly reduce environmental hazards.

In this work, we investigated the efficiency of pollutant photodegradation by 3D-printed photocatalysts containing nano-metal oxides, using aquatic ecotoxicological assays representing three trophic levels. We assessed the biological effects of the nano-metal oxides themselves, the photocatalysts produced from them, and the residues originating from the degradation of rhodamine B and paracetamol using the *Aliivibrio fischeri* cytotoxicity test, the *Daphnia magna* immobilization assay, and the zebrafish (*Danio rerio*) embryo (ZETA) test. This integrated approach enabled us to evaluate both the direct toxicity of the materials and the ecological relevance of the treated effluents.

Our results clearly showed that nano-metal oxides exhibit toxicity toward all test organisms, whereas the 3D-printed structures exerted substantially lower effects, indicating that sintering reduces the biological impact of the catalytic components. For rhodamine B solutions treated with the printed structures, a marked decrease or even complete elimination of toxicity was observed, confirming effective detoxification alongside photodegradation. These metal-oxide composite-based structures therefore mitigate the negative effects associated with metal-ion release and efficiently photooxidize and detoxify micropollutants. Overall, our findings highlight the importance of combining advanced treatment technologies with ecotoxicological

assessment to ensure that wastewater treatment processes targeting micropollutant removal are both effective and environmentally safe.

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3.27.P-Mo255 PFAS in Brazilian Citrus Commodities: Levels, Sulfluramid as a Key Precursor, and Human Health Risk Implications

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Brazil is the world's largest consumer of pesticides, and sulfluramid (EtFOSA) is extensively used to control leaf-cutter ants. Its aerobic degradation produces perfluorooctanesulfonic acid (PFOS) and, through indirect aqueous photolysis, perfluorooctanoic acid (PFOA), two persistent and bioaccumulative per- and polyfluoroalkyl substances (PFAS). Despite evidence that PFAS can migrate through soils, water, and food webs, data on their occurrence in tropical agricultural commodities and transfer across interconnected environmental and biological matrices remain limited. Within a One Health framework, assessing PFAS exposure is critical, as it integrates environmental persistence, crop uptake, food and water contamination, and potential impacts on human and animal health. High exposures to PFOS and PFOA have been linked to immunotoxic, carcinogenic, metabolic, and developmental effects. Yet, dietary exposure data from major food-producing regions in the Global South are scarce.

This study developed an analytical methodology for detecting PFOS and PFOA, originating directly or via sulfluramid degradation, in Brazilian citrus commodities. Using ultra-high-performance liquid chromatography coupled to sequential mass spectrometry (UPLC-MS/MS) with QuEChERS sample preparation, 60 citrus samples from São Paulo were analyzed, the state that represents approximately two-thirds of the planting territory and three-quarters of the production of this citrus fruit of Brazil. The calibration curve ranging from 0.001 to 100 ng/mL. PFOA was detected in 100% of samples, and PFOS was the second most prevalent compound, while sulfluramid was detected in some samples, likely due to rapid transformation into its degradation products. Estimated daily intake, hazard quotient (HQ), and cancer risk (CR) assessments revealed $HQ > 1$ and $CR > 10^{-6}$ for PFOA, highlighting potential health risks for domestic consumers and international importers.

The methodology proved reliable and reproducible despite analytical challenges, providing crucial data from an underrepresented region. These findings underscore the need for regulatory monitoring of PFAS in Brazilian food and contribute to understanding PFAS dynamics across environmental, animal, and human health interfaces under the One Health paradigm.

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3.27.P-Mo256 Children's Exposure to Airborne Chemicals in Danish Schools and Homes

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Indoor air quality assessments often overlook chemical mixture complexity, limiting comprehensive health risk evaluations for vulnerable groups like children. This study addresses this gap by analysing fluorotelomer alcohols (FTOHs; known PFAS precursors), biocides, plasticisers, polycyclic aromatic hydrocarbons (PAHs), and brominated and organophosphorus flame retardants (BFRs and OFRs) in air and dust samples from Danish

homes and schools. The chemical data is integrated with results from a walkthrough survey for the characterisation of classrooms, buildings, and surroundings.

Samples were collected at five schools, one school gym, and five homes between October 2024-2025, with two sampling periods per site. Air samples were collected using low-volume pumps (4.0 L min⁻¹) during school hours and overnight/weekends in homes, with dual Isolut® ENV+ SPE cartridges. After sampling, cartridges were spiked with labelled standards and extracted using group-specific solvents. Extracts were analysed for FTOHs and BFRs by GC/MS, biocides by LC-MS/MS, PAHs by GC-MS/MS, and OFR and plasticisers by LC-MS/MS coupled with turbulent flow chromatography (TFC) for online purification. Dust was collected via gravitational settling over five months and by vacuum sampling.

Preliminary data show the presence of FTOHs in all air samples, dominated by 6:2 and 8:2 homologues.

Concentrations ranged between 258–10,959 pg m⁻³ in schools, with homes showing significantly higher levels (up to 49,641 pg m⁻³), likely due to lower nighttime ventilation and more emission sources. PAHs also showed higher levels in homes (1,694–104,398 pg m⁻³) compared to schools (1,701–41,358 pg m⁻³). Five of the 12 biocides were detected in air, with DEET reaching 215,472 pg m⁻³ in one school sample. Other biocides appeared less frequently and at lower levels (5–648 pg m⁻³). BFRs were below detection limits in air. Analysis of the other chemical groups is ongoing, and dust samples are being processed.

These preliminary findings confirm high indoor chemical concentrations in some Danish homes and schools. Elevated FTOH and PAH levels in homes highlight ventilation and emission source roles. The results support comprehensive risk assessments that consider combined chemical burdens rather than individual compounds. By integrating chemical data with environmental and behavioural walkthrough surveys, this study aims to guide evidence-based exposure reduction strategies, emphasising emerging pollutants.

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3.27.P-Mo257 Robust Methodology for Hyperspectral Characterization of PFAS in Plant Matrices Using AI

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Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants with severe environmental and health implications. Traditional detection methods are costly and spatially limited, creating an urgent need for scalable, non-invasive solutions. This study has developed a robust methodology for hyperspectral characterization of PFAS in plant matrices (focus on *lactuca sativa*), enabling indirect detection of soil contamination through vegetation.

The central hypothesis was that PFAS uptake induces physiological stress in plants, altering leaf optical properties and generating distinctive hyperspectral patterns. To test this, lettuces (*Lactuca sativa*) have been grown under controlled hydroponic conditions with graded PFAS concentrations and extracted by means of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Physiological indicators (e.g., stomatal conductance, chlorophyll fluorescence) have been monitored alongside hyperspectral signatures across VIS–NIR and red-edge regions. These data have been used to train AI models to identify spectral markers linked to PFAS accumulation.

The outcomes include validated algorithms capable of discriminating PFAS-induced spectral changes from other stressors, ensuring specificity and robustness. The methodology could be scaled to UAV and satellite imagery for operational monitoring. This approach addresses a critical knowledge gap by linking PFAS bioaccumulation to plant spectral response, offering a transferable protocol for precision agriculture and environmental surveillance. Its relevance lies in reducing monitoring costs, enabling dynamic risk mapping, and supporting regulatory compliance in sensitive areas. By integrating hyperspectral sensing with AI-driven analytics, this research advances remote contamination detection and strengthens strategies for sustainable soil management and food safety.

3.27.P-Mo259 PFAS in Elemental Analysis: Towards a Routine Screening Method for Total Fluorine

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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. Among other analytical challenges, the feasibility of the approach is heavily dependent on fast screening methods as over 10,000 substances fit the common PFAS definitions. Here, elemental analysis of fluorine can serve as an effective tool. By careful consideration and selection of methods like Graphite-Furnace Molecular Absorption Spectrometry (GF-MAS) or Combustion Ion Chromatography (CIC) as well as suitable sample preparation methods, several sum parameters can be determined, offering flexibility and applicability to a wide range of samples and matrices.

Two major analytical challenges were addressed in this study. First, the differentiation between inorganic and organic fluorine species was investigated by applying mass balancing approaches and selective enrichment. Second, species-specific response behaviors in fluorine quantification were eliminated through systematic comparison of digestion methods. This is particularly important for samples with unknown fluorine species composition. The most effective digestion approach was implemented in a semi-automated system, reducing manual workload and delivering substantial time savings while maintaining analytical reliability.

3.27.P-Mo260 Assessment of Health Risks from Polycyclic Aromatic Hydrocarbons and Their Halogenated Derivatives in Residential Indoor Environments

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Polycyclic aromatic hydrocarbons (PAHs) and their halogenated derivatives (XPAHs) are emerging persistent organic pollutants with potential carcinogenicity. Their occurrence in indoor environments remains largely unexplored. We measured 26 PAHs and 40 XPAHs in indoor air and house dust from 45 residences in Toyama, Shizuoka, and Kanagawa, Japan (2022–2024). XPAHs were detected at all sites, with concentrations 10^3 – 10^4 times lower than PAHs but with similar size distributions and compositions, suggesting analogous atmospheric behavior. Homes using unventilated wood stoves exhibited indoor/outdoor ratios >10 for high-molecular-weight PAHs, notably benzo[a]pyrene, indicating strong indoor sources. Cancer risk assessment showed inhalation as the dominant exposure pathway, with 95th percentile incremental lifetime cancer risk exceeding 1×10^{-6} in several areas, surpassing safety thresholds. These results demonstrate that indoor exposure to PAHs and XPAHs may pose non-negligible health risks and underscore the need for mitigation strategies, such as PM_{2.5} reduction, to protect public health.

3.27.P-Mo261 Towards accurate estimation of extractable (organic) fluorine (EOF)

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Given the complexity and abundance of fluorinated organic compounds in the environment, broader analytical methods like extractable organic fluorine (EOF) and adsorbable organic fluorine (AOF) with combustion ion chromatography, are now commonly employed to assess contamination levels across various matrices. Although it is well-known that fluoride in sample extracts can lead to overestimation of EOF as the measurement does not inherently distinguish between organically bound fluorine and inorganic fluorine (i.e. fluoride), it is still largely neglected primarily due to lack of adequate analytical approaches. In this study, we investigated the presence of fluoride (F-) in EOF-extracts and designed and validated a method for fluoride determination in small aliquots (~250 µL) of complex aqueous- and solvent matrices. The method is based on derivatization of fluoride to triphenylfluorosilane (TPSiF) with gas-chromatographic tandem mass spectrometry analysis (GC-MS/MS). Calibration was linear from 1–1000 µg/L ($R^2 > 0.99$) and the limit of detection (LOD) and quantification (LOQ) was 0.98 and 4.5 µg F-/L respectively. The relative standard deviation (RSD) was below 7% across the calibrated range. Certified reference material (CRM River water) and fluoride spiked minced meat were used to assess fluoride recovery to $103 \pm 3\%$ ($p = 0.10$) and $95 \pm 6\%$ ($p = 0.31$) of certified/spiked concentrations. Analysis of sample extracts showed that (1) solid phase extraction (SPE)-extracts of river water, (2) solvent extracts from aqueous-film-forming foam (AFFF)-impacted soil, and (3) extracts of a fish sample contained 29–49, 61 ± 4.7 , and 17–90 µg F-/L respectively. F- accounted for 3–59% of the measured EOF, indicating substantial overestimation. Therefore, the analysis and discount of F- in EOF-extracts is essential for accurate EOF determination by CIC. This study highlights the risk that past analyses may have overestimated EOF concentrations, in some cases as much as by 750%. Mitigating such large biases are important for accurate monitoring of environmental concentrations and for the evaluation of remediation strategies to remove PFAS in soils and waters, particularly in light of current regulatory development concerning EOF and PFAS. As such, we recommend that F- determinations of extracts are routinely employed to improve accuracy of the fluorine mass balance calculation and avoid overestimation of the EOF-content.

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3.27.P-Mo262 Ecotoxicogenomic Evaluation of the Photodegradation Products of Acesulfame-K

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Sugars provide essential dietary energy, but excessive intake is linked to several cardiometabolic conditions, prompting increased use of low-calorie artificial sweeteners. Acesulfame-K (Ace-K) is highly stable and minimally metabolized, leading to its frequent detection in aquatic environments, especially downstream of wastewater treatment plants. This persistence raises concerns about the formation of photodegradation products, such as sulfamic acid, during ultraviolet disinfection or from sunlight exposure. Although sulfamic acid is used in various household and industrial applications, its presence in surface waters may pose ecological risks. This study assessed the formation of Ace-K photodegradation products under simulated sunlight and UV irradiation. Also, acute toxicity was assessed using *Danio rerio* embryos and *Daphnia magna* according to the Organisation for Economic Cooperation and Development (OECD) test guidelines. Thereafter, transcriptome responses were evaluated to identify sub-lethal and molecular-level impacts. For the photodegradation experiments, Ace-K at pH 4, 7, and 9 was irradiated with simulated sunlight (>290 nm) for 20 days or UV light (254 nm) for 30 minutes. Samples were analyzed to quantify Ace-K and detect transformation products. For ecotoxicogenomic tests, *Daphnia magna* and *Danio rerio* embryos were exposed to fully degraded Ace-K and its known transformation product; sulfamic acid. RNA was extracted and sequenced. Statistically significant differentially expressed genes (DEGs) were identified using DESeq2, followed by over-representation analysis (ORA) using clusterProfiler. In this study, Ace-K was found to be stable in the UVB/UVA and visible light spectrum (>290 nm) but it is rapidly degraded under UVC, forming some transformation products such as hydroxyl-acesulfame and sulfamic acid. Ecotoxicology tests on *Danio rerio* revealed no significant morphological effects at low (10 µg/L) or high (100 mg/L) exposures. Ongoing molecular analysis will further explore potential effects on gene expression. Further insights are to be gained through the ecotoxicology experiments with *Daphnia magna*. These findings improve understanding of Ace-K environmental fate and highlight the importance of considering transformation products in risk assessment.

3.27.P-Mo263 Leveraging AFFFs to support suspect screening of PFAS in marine biota of low trophic level

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals extensively used for their unique properties, which have led to widespread environmental contamination, including in marine ecosystems. Notably, a wide range of PFAS is used in aqueous film-forming foam (AFFF) formulations, which are well-known sources of PFAS contamination. This study explores the use of AFFFs as qualitative reference materials to support suspect screening of PFAS in organisms of low trophic level.

Mussels and oysters were sampled in 2021 from 23 sites along the French coast. Samples were extracted using methanolic potassium hydroxide and purified through solid-phase extractions (WAX and ENVI-Carb-SPE). Analyses were performed using a LC-QTOF (Agilent Technologies). Suspect screening was conducted using EI-MAVEN software and an in-house suspect list of 866 PFAS. To enhance detection and annotation, 14 different AFFFs were analyzed as reference materials. Two types of AFFFs were included: FT-AFFFs, which contain mainly polyfluoroalkyl compounds, and ECF-AFFFs which predominantly contain perfluoroalkyl compounds.

Overall, 79 PFAS from 29 chemical classes were detected including non-biotransformed precursors. Most of the compounds detected in the bivalves were also detected in AFFFs. This work demonstrates the effectiveness of using AFFF formulations as reference material in PFAS suspect screening workflows. Co-analysis with biological extracts greatly assisted peak attribution, as a high number of features is found in these complex matrices increasing the likelihood of false-positive annotations. Applied to low trophic level species, such as mussels and oysters, this approach enhanced the detection of precursor compounds that might otherwise be biotransformed and undetected in higher trophic levels. In this study, AFFF played a key role by helping recover

low-intensity signals in biota samples, thereby improving detection sensitivity. In several cases, they were also essential for distinguishing between candidate isomeric structures, either with the characteristic FT-AFFF/ECF-AFFF composition pattern, or by providing key MS/MS information. This methodology highlighted numerous unregulated and not routinely monitored PFAS, some of which are, to the best of our knowledge, reported for the first time in marine organisms (e.g., X:2 FTSAm). The approach presented here offers a practical and informative strategy for expanding PFAS monitoring efforts in marine environments.

3.27.P-Mo264 Nontarget screening workflow for the analysis Per-and-polyfluoroalkyl substances in environmental samples using a benchtop multi-reflecting Time-of-flight mass spectrometer

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The widespread use of per and polyfluoroalkyl substances (PFAS) in industrial processing and manufacturing over the last decades has led to global environmental and health concerns. As the number of these compounds continues to increase, it is becoming a challenging task to monitor PFAS using the traditional targeted methods. A screening workflow using High Resolution Mass Spectrometry (HRMS) is an ideal bridge for monitoring the regulated PFAS and detecting non-regulated PFAS.

Here, PFAS content in river, biosolid and river leachate was determined. PFAS extractions were performed following the EPA 1633 guidance. 2 µL of both samples and standards were analyzed using an ACQUITY™ Premier UPLC™ system coupled to a benchtop multi-reflecting time-of-flight (MRT) mass spectrometer (Xevo™ MRT). Data were acquired in negative ionization mode at 20 Hz scan speed, using MSE, a data independent mode of acquisition.

The levels of sensitivity and dynamic range were evaluated for at least 30 PFAS standards. Non-Target screening (NTS) workflow within waters_connect software along with the PFAS libraries (in-house Waters generated and an online version of the NIST PFAS library), enabled the identification and quantification of at least five compounds with a mass measurement of <500 ppb and mass resolution of >60,000 FWHM, in river, biosolid and leachate samples. The samples tested contained varying amounts of PFAS.

The work described highlights the exceptional mass accuracy, sensitivity, high mass resolution, and dynamic range of the Xevo MRT MS system for PFAS analysis. For most of the tested compounds the limits of detection were in the low ng/L range (< 5 ng/L). The dynamic range spanned at least four orders of magnitude from 1 to 10,000 ng/L. The screening workflow is based on accurate mass, fragment ion information, isotopic matching and mass defect, this coupled with high mass resolution, fast scan speeds and exceptional levels of mass precision of the. Using the NTS workflow allowed the detection of PFAS compounds that were not incorporated into the PFAS libraries. Xevo MRT mass spectrometer significantly reduces false positives and enables rapid, confident assignment and identification of PFAS related compounds.

Disclaimer/Disclosure:

There are no conflicts of interest.

3.27.P-Mo265 Concentrations and Profiles of Per- and Polyfluoroalkyl Substances (PFAS) in Dust and Drinking Water from the United Kingdom: Implications for Human Exposure

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Per- and polyfluoroalkyl substances (PFAS) have found extensive application in a wide range of manufacturing industries and commercial products due to their water and grease proofing properties. Several legacy PFAS have been listed as persistent organic pollutants (POPs) for restriction and ultimate elimination under the Stockholm Convention, leading to the introduction of a growing number of novel PFAS as substitutes. However, both the extent of novel PFAS contamination in indoor environments of the United Kingdom (UK) and the associated exposure levels of the population are still poorly understood. Therefore, our study aims to explore a suite of 44 PFAS in indoor dust and drinking water and to conduct a preliminary exposure and risk assessment of the UK population to PFAS. Indoor dust and drinking water samples were collected from households and offices in the West Midlands, UK, followed by quantitative analysis using ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. PFAS were detected in all samples, with the median total concentration of 114 and 84.8 ng/g in home dust and office dust, 2.26 ng/L in household tap water and 1.54 ng/L in office drinking water, respectively. The estimated daily intakes of PFAS through dust digestion and water consumption were 0.07 and 0.14 ng/kg body weight/day for adults and 0.42 and 1.34 ng/kg body weight/day for toddlers, under average and high exposure scenarios, respectively. Household tap water

represented the primary contributor to the median daily PFAS intake for adults (54.4%), whereas home dust ingestion was the dominant source for toddlers (69.9%). Our study highlights the widespread occurrence of legacy and novel PFAS in the UK indoor environment and underscores the need for continued monitoring and prompt regulatory actions to reduce the risk of PFAS to human health and the UK environment.

3.27.P-Mo266 PFAS Measurements in Complex Environmental Samples (Biosolids, Soil, Sediment, Wastewater) with Enhanced Matrix Removal and Automation

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Recent method development efforts to measure PFAS compounds in various sample matrices led to testing standards including but not limited to those published by USEPA, ASTM, ISO and many other agencies and institutions. PFAS testing has evolved to measure down to ng/L (or pg/L) in drinking water and ng/mg in other solid environmental samples. While expanding testing protocols to more complex matrices outside drinking water samples, one of the most challenging aspects of the analytical workflow lies in the effectiveness of sample matrix cleanup. Matrix interferences coming from the complex sample matrices can not only damage the instruments requiring more frequent maintenance on them, but also inconsistent data can result in false positives/negatives.

To address this issue for complex environmental sample preparation, greatly enhanced cleanup approach is applied streamlining the workflow and minimizing human errors resulting in consistent data meeting USEPA 1633A requirements with soil and biosolids

3.27.P-Mo267 Investigating the Organofluorine Load in a UK Wastewater Treatment Plant

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Poly- and Perfluoroalkyl Substances (PFAS) are a diverse 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 and ubiquitous occurrence, has led to significant regulatory responses to manage PFAS pollution by replacing legacy PFAS with novel alternatives. Eventually, these substances find their way into the environment throughout their life cycle from manufacture to waste disposal. PFAS and other fluorinated substances enter wastewater and runoff streams and eventually end up in rivers and estuaries. However, PFAS represent only a fraction of the organofluorine present in wastewater with other sources including pharmaceuticals, pesticides and polymer-derived fragments. Because many of these substances lack standards and established analytical methods, targeted methods likely underestimate the amount of organic fluorine burden in WWTPs. To address this a combined mass balance approach integrating targeted analysis, Extractable Organic Fluorine (EOF) and Total Fluorine (TF) was utilised to investigate the organofluorine loads in a UK WWTP. Diffusive gradient in thin films (DGTs) passive samplers were deployed across treatment stages and sludge samples were collected to characterise the extractable organofluorine in the aqueous and solid compartments, respectively. Initial results showed that PFAS accounted only for 8% of the EOF for the aqueous compartment, while sludge-extractable organofluorine accounted only 0.2% of the TF. These findings reveal that the vast majority of the organofluorine in the WWTP is unidentified demonstrating the need for future developments of new holistic and sensitive analytical methods to better characterise the organofluorine burden in WWTPs.

3.27.P-Mo268 PFAS in Air: Regulatory and Standards Landscape, Method Development, and Workflow Guidance

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PFAS (per- and polyfluoroalkyl substances) are a group of highly persistent chemicals that are now recognized as a global concern. While much attention has focused on water and soil, PFAS can also be emitted into the air during industrial processes, product use, and waste handling. Once airborne, they may travel long distances, making air a critical pathway for dispersion and exposure.

Testing for PFAS in air is challenging. They can exist both as vapours and bound to particles, they occur at very low concentrations, and background contamination can easily compromise results. For industry and environmental consultants tasked with source emission testing and environmental monitoring, understanding these challenges and the expectations of regulators is essential.

This presentation is designed to give a clear overview of the current landscape for monitoring PFAS in air. It will outline practical considerations for sampling and analysis, highlight the importance of contamination control, and provide updates on U.S. and international efforts toward method development, standardization, and regulatory guidance. Example workflows using thermal desorption coupled with gas chromatography-mass spectrometry (TD-GC-MS) will also be discussed, showing how these techniques can be applied to real-world testing of PFAS in air.

3.27.P-Mo269 Hydrodynamic Modelling of Pharmaceuticals and PFAS in a Swedish Lake: Spatiotemporal Variations

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Human-derived micropollutants such as active pharmaceutical ingredients (APIs) and per- and polyfluoroalkyl substances (PFAS) are continuously released to surface water sources, mainly via wastewater and riverine inputs. Conventional monitoring typically provides sparse data in space and time, making it difficult to characterise concentration dynamics and to support risk assessment and drinking water protection. This work addresses the challenge of predicting spatiotemporal variations in micropollutant concentrations at the scale of whole lakes. We develop and apply a modelling framework that combines emission modelling from human activities with three-dimensional hydrodynamic transport modelling, and evaluate it for APIs and PFAS. The hydrodynamic model was set up for Lake Ekoln in Sweden, and validated against observed water temperatures. For APIs, emissions from a wastewater treatment plant were estimated from prescription data for >500 substances and coupled to the hydrodynamic model. The emission estimates were validated against monthly effluent measurements of 10 APIs; 102 of 103 measured concentrations were predicted within a factor of 10. Modelled lake concentrations were compared with 321 historical measurements of 20 APIs; 73% and 99% of predictions were within a factor of 10 and 100 of observations, respectively. For PFAS, emissions from the wastewater treatment plant and the lake's main inflow were represented in the model based on available measurements, and the simulated lake concentrations were generally consistent with the limited observational data. The modelling results confirmed that the main inflow to the lake is the dominant PFAS source. The model revealed pronounced seasonal and spatial variability. Our results demonstrate that relatively simple emission estimates, when combined with hydrodynamic modelling, can reproduce observed micropollutant patterns and reveal concentration gradients that routine monitoring would miss. This approach can guide targeted monitoring, inform mitigation of dominant sources, and support risk management for aquatic ecosystems and drinking water sources.

3.27.P-Mo270 Enhanced performance for FOSA and FOSEs analysis with an ultra-sensitive PFAS-multiclass method in environmental samples.

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With the increasing regulatory drivers and public pressure for managing PFAS in the environment, foods, and consumer and industrial products, laboratories are in need of efficient multiclass-PFAS methods that enable routine ultra-sensitive quantitation. As the classes of PFAS to be monitored expand the instrument performance may be compromised for specific compounds as their ionization efficiency changes based on their physico-chemical properties. In this work we validated a LC-MS/MS method (LCMS-8065 XE, Shimadzu Corporation) for a multiclass-PFAS panel including PFCAs, PFSAs, FTSSs, FOSAs, FOSAs, FOSEs, FTCAS, PFESAs and PFECAs across matrices of environmental relevance (drinking water, wastewater, tissue...). Sample preparation varied depending on the matrix of interest: co-solvation and direct injection for drinking water, and pre-concentration (either Solid Phase Extraction or solvent extraction) for other sample types. Method Detection Limits (MDLs) were below 1 ng/L in drinking water for all targeted compounds. In solid samples, MDLs ranged from <0.05 to 0.2 ng/g for PFCAs, PFSAs, PFESAs and PFECAs, < 0.5 ng/g for FTSSs and FTCAS, and < 0.6 ng/g for FOSAs, FOSAs, and FOSEs. In this presentation we will share the detailed results of the method validation, with emphasis on classes of PFASs that are historically worse performers in multiclass-PFAS methods, such as FOSAs and FOSEs.

3.27.P-Mo271 Analysis of Neutral PFAS in Ambient Air Using Thermal Desorption Gas Chromatograph-Mass Spectrometry.

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In response to the tightening of PFAS regulations, analytical methods have been developed targeting non-

volatile (ionizable) PFASs for various matrices such as water, soil, and food using liquid chromatography-mass spectrometry (LC/MS/MS). However, for the analysis of semi-volatile or volatile neutral PFAS gas chromatography-mass spectrometry (GC/MS) is the most effective technique.

In this study, nine semi-volatile and volatile neutral PFASs including four fluorotelomer alcohols (FTOHs), three fluorotelomer acrylates (FTACs), and two perfluoroalkyl sulfonamides (FOSAs), were targeted with a method for air samples using thermal desorption GC-MS (TD-GC/MS), without the need of solvent-based sample extraction.

A TD-GC/MS (GCMS-QP2020 NX coupled with TD-30R, Shimadzu Corporation). A preliminary study was conducted to select the most suitable sample tubes: Tenax TA and Tenax TA/Carboxen 1000 were evaluated. A mix of the targeted compounds were spiked onto the sample tube to achieve 0.05 to 12.5 ng of each compound, and the sample was purged with nitrogen gas to be used for the calibrations. Based on the results from the sample tube evaluation, the two-layer type sample tube of Tenax TA/ Carboxen 1000 was selected for further method evaluation. For the recovery test using air samples, 4 FTOHs were added to the sample tube at 10 ng, 3 FTACs and 2 FOSAs at 1 ng, and 20 L of air was collected at 100 mL/min. The measurement of standard samples for the nine PFAS components showed that for all compounds, measurement was possible from 0.05 ng, and good linearity was confirmed in the range from 0.05 to 12.5 ng with a correlation coefficient of $R > 0.998$ for the calibration curve. Furthermore, the area value and concentration %RSD ($n=3$) < 6 at 0.2 ng confirmed good reproducibility. The results of the additive recovery test using air samples showed that the additive recovery was 77 to 106 % and the concentration %RSD ($n=3$) < 8 , which were generally good results. In this presentation we will share the detailed results from the evaluation of different sample tubes and the overall performance of the TD-GC/MS method developed in this work for the simultaneous analysis of FTOHs, FTACs and FOSAs in air samples.

3.28 Trifluoroacetic Acid in the Environment – Here Today, More Tomorrow

3.28.T-01 Atmospheric passive sampling of gaseous perfluorinated carboxylic acids dominated by trifluoroacetic acid (TFA)

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Atmospheric oxidation of volatile perfluorinated alkyl substances (PFAS) is a major source of gaseous perfluorinated carboxylic acids (PFCAs). Their abundance and deposition after formation controls their subsequent impacts on the global environment. For example, atmospheric formation leads to observed parts-per-trillion mixing ratios of TFA in the gas phase, as well as $\mu\text{g per L}$ concentrations in Arctic ice caps. There are numerous precursors that can form mostly TFA, but also other short chain PFCAs (C_6 or smaller) through atmospheric oxidation, including chlorofluorocarbon replacements.

Active atmospheric sampling methods for PFCAs have the potential for substantial analytical artifacts and are yet to be subject to rigorous validation experiments, such as calibration with gaseous analytes or method intercomparisons. Passive sampling methods have similar limitations and have treated PFCAs as traditional persistent organic pollutants instead of targeting their acid functionalities to elicit method specificity.

As a result, insights into the abundance, phase distribution, and spatial and temporal variability of atmospheric PFCAs has been limited. Here we demonstrate a calibrated passive sampling technique for gaseous PFCAs based on long-established acid sampling strategies using nylon filters in a low cost diffusion-driven sampler. These were deployed in several large field campaigns in Canada from weeks to months timescales to study controls on the formation and fate of TFA and other atmospheric PFCAs.

In nearly all cases, TFA was the most abundant of the analyzed homologue suite, present at hundreds of parts per quadrillion to parts per trillion by volume mixing ratios (ppqv - pptv). When measured with seasonal time resolution, TFA abundance increased when the abundance of atmospheric precursors and oxidants are also expected to maximize. When measured with spatial resolution, heterogeneity in TFA levels was observed within urban landscapes and along urban-to-rural transects, suggestive of hotspots for direct emissions or atmospheric transport. Measurements we conducted within a wastewater treatment plant found TFA as the most abundant gaseous PFCA and prior knowledge of these facilities as point sources in the urban environment. The emerging landscape of atmospheric TFA will be discussed alongside limitations and knowledge gaps from these first measurements across a wide variety of atmospheric environments.

3.28.T-02 Advancing Our Understanding of the Sources of Atmospheric TFA

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Atmospheric formation is a major source of perfluoroalkyl carboxylic acids (PFCA) to the environment. The number of potential fluorinated precursors is inversely related to chain length, with the shortest PFCA, trifluoroacetic acid (TFA), having numerous potential precursors. Atmospheric formation can explain observed levels of TFA in diverse locations, including urban air Arctic ice caps. There are numerous precursors that can form TFA through atmospheric oxidation, including chlorofluorocarbon replacements, such as hydrofluorocarbons (HFCs) and hydrofluoroolefins (HFOs). While certain mechanisms that form TFA as a major product have been well studied (e.g., HFC-134a), there has been less focus (and a great deal of confusion) on mechanisms that form TFA and as minor products. For example, HFC-125 (CF₃CHF₂) has been the subject of conflicting reports on its TFA formation. This confusion stems, in part, from the dependence of the yield on nitric oxide (NO) and peroxy radical (RO₂) ratios. In addition, formation of TFA is typically reported as a molar yield of the precursor product. This chemical yield is not readily digestible by non-chemists. With a detailed re-evaluation of the chemistry that forms TFA from precursors, here we demonstrate that we can more accurately predict the formation of TFA. We also propose a new metric, the atmospheric PFCA formation potential (APFP) that can be used by policymakers. This mass-based definition is more consistent with other metrics used by policy makers, including the global warming potential. This new metric allows for better assessment of the overall impacts of TFA precursor chemicals. For example, this allows emissions of precursor chemicals, typically reported in mass units, to be directly related to the mass of TFA that will be formed from their ultimate atmospheric degradation. In addition, the trade-offs between climate and TFA formation can be readily assessed. Examples of how the APFP can help in our evaluation of precursor usage will be presented.

3.28.T-03 Rising TFA Concentrations in Danish Groundwater: Atmospheric and Agricultural Sources

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TFA is widespread in the environment, including in groundwater, where it is found in concentrations hundreds or even thousands of times higher than any other PFAS. Due to its high chemical and biological stability and its low sorption to soil, TFA can be considered to move conservatively with groundwater. Under these assumptions, we have demonstrated that the concentration of TFA in groundwater has risen from non-detectable levels in Danish groundwater formed before 1960 to detectable levels in all Danish groundwater formed after 1980, with a consistent increase that appears to be ongoing.

There are likely two major sources contributing to this rise in TFA, and in both cases TFA is a degradation product—either from fluorinated gases in the atmosphere or from pesticides used in agriculture. In Denmark, the latter source has been considered somewhat hypothetical, since only a few pesticides have been shown to form TFA during degradation, none of which have been used in Denmark. We therefore conducted a degradation study using seven common pesticides that contain a C-CF₃ group, all of which were found to form TFA in three agricultural soils.

Taken together, the available evidence suggests that rising atmospheric input currently contributes approximately 0.5 µg/L TFA to Danish groundwater. In areas with conventional agriculture, there is an additional TFA input from pesticides, with a magnitude similar to or greater than the atmospheric input. This source has also been increasing over the past decades but is expected to decline in the future due to a recent, Danish ban on many C-CF₃ pesticides.

The results of our work can inform ongoing discussions about regulations of fluorinated gases and pesticides, as well as discussions about future legal threshold values for TFA in the environment and in drinking water.

3.28.T-04 Widespread Trifluoroacetic Acid (TFA) Pollution in UK Surface Waters

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Trifluoroacetic acid (TFA; CF₃COOH) is now recognised as the most abundant of per- and polyfluoroalkyl substances (PFAS) in the global environment. Its formation from longer-chain PFAS, combined with extreme solubility and mobility, leads to diffuse inputs to aquatic systems from multiple pathways. Despite international evidence of increasing TFA concentrations in water systems, and growing concern over potential health and environmental impacts, our work is the first to determine the presence and potential drivers of TFA pollution in UK surface water.

We quantified TFA in repeat samples collected from 54 river monitoring sites (214 total samples) spanning a

broad range of UK catchments and supplemented this with 19 sites in Yorkshire, where monthly composite samples were collected using automatic samplers (268 total samples). TFA concentrations were determined by direct-injection (40 µL) high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS). We extracted environmental variables for each of the catchments, including the percentage of arable land, population sum and exposure to landfill sites, military bases and airfields, golf courses, industrial sites and wastewater treatment plants. We examine how these variables relate to TFA flux via General Additive Modelling (GAM).

TFA was detected at 97% of sites during the national sampling campaign, and in 99% of samples in the regional campaign, demonstrating widespread contamination across the UK. We recorded the second-highest level of TFA pollution ever reported in surface water globally (River Kelvin, Glasgow), while the mean UK concentration of 2,211 ng/L aligns with observations from other European and international studies. Further analysis has highlighted the percentage of arable land within the catchment (EDF = 2.1, $p < 0.01$) and exposure to golf courses (EDF = 1.463, $p < 0.001$) as two significant non-linear predictors of TFA flux.

This first national baseline of TFA in UK surface waters reveals widespread contamination, underscoring the chemical's persistence and suggests substantial inputs from TFA precursors across UK catchments. Our review of environmental variables aims to shed light on potential influences of TFA pollution such as industrial pollution, wastewater treatment, and use of PFAS pesticides. Our findings highlight the need for strengthened evaluation and control of TFA and wider PFAS-precursor emissions in the UK.

Disclaimer/Disclosure:

This research was completed by the University of York and Fidra. Fidra is an endowment funded SCIO and Scottish registered charity (SC043895). Fidra's accounts are published on the OSCR website.

3.28.T-05 Trifluoroacetic Acid Formation in Potato Cultivation: Impact of Fluazinam, Fluopicolide and Oxathiapiprolin Degradation

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Trifluoroacetic acid (TFA) is a highly persistent and mobile compound formed as a quasi-terminal degradation product of substances containing trifluoromethyl groups (CF₃). These moieties are common in agrochemicals, making pesticides a significant source of TFA, often referred to as “PFAS pesticides.” TFA is increasingly detected in environmental and food matrices, raising concerns about its accumulation and potential risks to ecosystems and human health. Despite its relevance, knowledge gaps remain regarding the fraction of applied pesticides converted to TFA and its distribution across soil, water, and crops under field conditions.

This study investigates TFA formation and fate in a multi-matrix field experiment involving potato cultivation with and without PFAS-containing fungicides. The trial was conducted at Lilla Böslid, Halland, Sweden, using twelve plots arranged in three treatments: untreated control, non-PFAS fungicides, and “PFAS pesticides” (fungicides fluazinam, fluopicolide and oxathiapiprolin). Sampling included drainage water, adjacent stream water, precipitation, irrigation, soil, foliage, and tubers over one growing season, with continued water and soil monitoring planned until spring 2026. Chemical analyses include TFA, four additional ultrashort PFAS, other PFAS and pesticide residues.

Preliminary results indicate background TFA concentrations in drainage water of $1,300 \pm 110$ ng/L, soil levels averaging 0.68 µg/kg dry matter and 35-80 µg/kg in foliage before pesticide treatment. Theoretical calculations suggest that full conversion of applied pesticides could yield TFA concentrations of 170,000 ng/L in drainage, highlighting its potential significance as a source. Further evaluation will address TFA distribution in crops, seasonal variation, and relative contributions from precipitation versus pesticide degradation. Additional objectives include constructing a CF₃-moiety budget and assessing potato yield and disease incidence. This research will provide critical insights into TFA dynamics in agriculture, supporting mitigation strategies and informing regulatory frameworks. Findings will help quantify the environmental burden of PFAS pesticides and guide sustainable crop protection practices.

3.28.P Trifluoroacetic Acid in the Environment – Here Today, More Tomorrow

3.28.P-We310 Occurrence of Trifluoroacetic Acid in Surface Water and Groundwater from Swedish Agricultural Areas

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Several active substances in plant protection products approved in the EU fall under the OECD definition of PFAS. Many of these PFAS pesticides can degrade to the ultrashort-chain transformation product trifluoroacetic acid (TFA), a very persistent compound. Because TFA originates from multiple environmental sources, including pesticides, its presence and potential accumulation in surface water and groundwater are of increasing concern.

This study aims to characterize the occurrence and concentrations of TFA in agricultural surface water and shallow groundwater in Sweden and to evaluate these concentrations in relation to ecological and groundwater quality thresholds.

Water samples collected in 2021, 2022 and 2025 within the Swedish national monitoring program were analyzed for TFA. In 2021-2022, 20 surface water samples and 12 groundwater samples were analyzed for a set of 67 pesticide metabolites, including TFA. In 2025, we collect 50 additional surface water grab samples (May–Oct, biweekly) and 40 groundwater samples from the same areas and analyze them for three ultrashort-chain PFAS, including TFA. The full dataset will include 70 surface water samples from long-term agricultural monitoring sites, 4 samples downstream of greenhouses and orchards, and 52 samples of shallow groundwater (2–8 m depth) from field-edge monitoring wells.

To date, 88 samples have been analyzed. TFA was detected in all surface water samples and in all but a few groundwater samples (reporting limit 0.05 µg/L). Surface water concentrations (0.38–2.4 µg/L) were well below the predicted no-effect concentration reported in the REACH registration dossier for TFA (560 µg/L), but relatively high compared to other detected pesticide metabolites in agricultural streams. Concentrations in shallow groundwater (0–5.4 µg/L) frequently exceeded the 0.1 µg/L threshold value applied to individual pesticides and their metabolites in groundwater, indicating a potential long-term contribution of agricultural recharge to TFA levels in groundwater. Detailed pesticide use data from the long-term monitoring catchments enable further assessment of possible sources of TFA.

Final results from the study will be reported to the Swedish Environmental Protection Agency in March 2026. Overall, this work advances understanding of the occurrence of TFA in agricultural landscapes and supports ongoing evaluation of pesticide-derived TFA and their relevance for groundwater risk and aquatic risk assessment.

Disclaimer/Disclosure:

The study was financed by the Swedish Environmental Protection Agency. Samples from 2021 and 2022 were analyzed by DVGW Technology Centre for Water (TZW), Karlsruhe, Germany. Samples from 2025 were analyzed by Eurofins.

3.28.P-We311 Occurrence of trifluoroacetic acid and other ultrashort-chain PFAS in groundwater

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Trifluoroacetic acid (TFA) is a very mobile and persistent perfluorinated compound. It is often present in water at concentrations orders of magnitude higher than those of other per- and polyfluorinated substances (PFAS) and is also increasingly being detected in drinking water. However, little is known about the occurrence of TFA and other ultrashort-chain PFAS ($C < 4$) in groundwater. In this study, existing and newly collected monitoring data were used to investigate the occurrence of TFA and other ultrashort-chain PFAS (Figure 1) in groundwaters characterized by different degrees of vulnerability. TFA was detected in 89, 41, and 15% of the sites from aquifers categorized as ‘vulnerable’ ($n = 74$), ‘moderately vulnerable’ ($n = 22$), and ‘not vulnerable’ ($n = 20$), respectively (Table 1). Six other ultrashort-chain PFAS mostly occurred below the limit of quantification (0.0005–0.002 µg/L), but PFPrA and TFMS were detected in 17 and 13% of the samples, respectively. TFMS was detected at levels < 0.060 µg/L, and PFPrA below 0.025 µg/L, respectively. Both TFMS and PFPrA occurred only in ‘vulnerable’ and ‘moderately vulnerable’ groundwaters. A correlation was found between the percentage of water younger than 25 or 50 years in groundwater sources and the concentration of TFA in the

abstracted groundwater ($r = 0.77$, with a few outliers excluded), indicating input from diffuse sources with a peak between 1975 and 2000. The highest concentrations were found at abstraction sites with a large proportion of infiltrated surface water. The contribution of TFA (and other ultrashort-chain PFAS) to the weighted toxicological impact as part of a mixture of various PFAS requires further investigation, as TFA and other ultrashort-chain PFAS pose a risk to groundwater quality.

3.28.P-We312 Monitoring Ultra-Short Chain PFAS in Flanders: Occurrence, Patterns, and Tracing TFA

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Ultra-short chain PFAS (USC PFAS, ΣC_3), such as trifluoroacetic acid (TFA), are characterised by high environmental mobility combined with strong persistence. To better understand their occurrence in Flanders, the Flanders Environment Agency conducted a large-scale monitoring campaign assessing USC PFAS in wastewater, surface water, and groundwater. In the initial campaign, 49 PFAS, including 7 USC PFAS, were investigated in wastewater and surface water. A second, follow-up campaign focused on USC PFAS in rural areas (with particular emphasis on TFA), with targeted sampling in surface and groundwater. In parallel, PFAS pesticides (i.e., plant protection products suspected of degrading into TFA) were also sampled to explore potential links with the environmental occurrence of TFA.

Based on the results of the first campaign, USC PFAS were widely detected across Flanders, with TFA present in 74% of wastewater and 88% of surface water sites. The USC PFAS in surface water showed consistently elevated concentrations in urban, rural and diffuse areas, indicating a widespread distribution throughout the region.

The second campaign similarly revealed a substantial contribution of TFA to the overall PFAS burden at rural sites. TFA was detected in 36 of 40 surface water sites and in 267 of 272 groundwater sites. The median TFA concentration in surface water was 2.3 $\mu\text{g/L}$, with peak values up to 13 $\mu\text{g/L}$. Among PFAS pesticides, fluopicolide and fluopyram occurred at the highest concentrations in surface water, with median values of 0.025 and 0.031 $\mu\text{g/L}$, respectively. In groundwater, the median TFA concentration was 2.8 $\mu\text{g/L}$ with a maximum of 30 $\mu\text{g/L}$. Detections of PFAS pesticides in groundwater were very low. Fluopicolide was detected the most (in 25 of the 272) and was quantified in the highest concentration (max. 1.5 $\mu\text{g/L}$).

Statistical analyses consistently showed a positive relationship between TFA and the pesticide fluopicolide in surface waters. The observed associations between TFA concentrations and specific crop types were weaker and should be treated as exploratory, given the absence of detailed pesticide use data. The monitoring dataset has some limitations, particularly the low sampling frequency, which prevents a reliable assessment of seasonal variability in TFA concentration or pesticide application patterns. Even so, the high detection frequency of TFA in surface and groundwater indicates a broadly diffuse occurrence across rural areas in Flanders.

Disclaimer/Disclosure:

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3.28.P-We313 TFA Analysis in Drinking Water

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Trifluoroacetic acid (TFA, CF_3COOH) is a low molecular weight perfluorinated carboxylic acid formed as a breakdown product of various industrial chemicals, including refrigerants and pesticides. Found increasingly in surface water, groundwater, and drinking water, it has raised environmental and health concerns due to its resistance to degradation and removal.

Toxicological concerns have prompted the European Food Safety Authority (EFSA) to propose an acceptable daily intake (ADI) of 0.03 mg/kg body weight per day, expressed as sodium trifluoroacetate.¹ This value is undergoing consultation¹ due to emerging data suggesting potential classification of TFA as a Reproductive Toxicant Category 1B and a very persistent, very mobile (vPvM) substance under REACH.

This poster describes a robust and ultra-fast method for the analysis of trifluoroacetic acid (TFA) in multiple water sources using the SCIEX 7500+ system, enabling high-throughput workflows with minimal sample handling. The method, which includes confirmation with both a quantifier and qualifier transition for confirmation, achieves a rapid turnaround time of just 4 minutes and 30 seconds between samples, allowing for the processing of 40 samples—including blanks and standards—in under four hours.

An injection volume of just 5 μL was required to achieve a lower limit of quantitation of 10 ng/L ensuring

sensitive detection with low contamination of mobile phases and a TFA-free blank when internal standard (IS) was excluded. The robust analytical method was shown with batch analysis of >90 successful injections without signal degradation or significant change in baseline noise.

Retention time stability across the sequence was excellent with a %RSD of 2.32% across the 71 injections. Internal standard recoveries were tracked across the entire sequence and were between 80 and 120% of the mean

3.28.P-We314 The Prevalence of Trifluoroacetic Acid in Drinking Water Supplies across England and Wales

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Trifluoroacetic acid (TFA), an ultrashort-chain perfluoroalkyl acid (PFAA), is a highly mobile and persistent chemical within the per- and polyfluorinated substances (PFAS) family. Recent studies have reported its widespread occurrence in environmental matrices at concentrations exceeding other PFAS compounds. The presence of TFA in drinking water is an emerging concern across Europe, with many regulatory bodies taking action to address the issue, however despite this threshold concentrations remain poorly defined and data on its prevalence is limited. This study, led by the UK's Department for Environment, Food and Rural Affairs (DEFRA) aims to address this knowledge gap by characterising TFA's occurrence in England and Wales drinking water sources.

Therefore, we conducted a literature review compiling existing data on prevalence and sources of TFA in this region. Spatial insights guided site selection for a targeted monitoring program at four drinking treatment works, enabling comparison of urban and rural catchments and allowing the evaluation of the prevalence of TFA across England and Wales. In addition, samples were taken at multiple stages of the treatment process (raw, partially treated, and final effluent) to assess the effectiveness of current and advanced treatment technologies in removing TFA.

Findings from the literature review found a notable lack of data on environmental prevalence in England and Wales, underscoring the need for targeted monitoring. This study therefore presents preliminary monitoring results from the first coordinated effort to map TFA in drinking water supplies across these regions. This will include the influence of different sources on TFA concentration, including those from local inputs such as pesticides, industrial runoff, and emissions from fluorochemical industries, as well as more diffuse contributions from the atmospheric degradation of HFCs or HFOs used in refrigeration and cooling systems.

Findings from this study will help identify drinking water catchments most at risk of TFA contamination, provide evidence on the effectiveness of current treatment processes in removing or degrading TFA, and highlight any unintended consequences within treatment systems that could further increase concentrations. Furthermore, the results will underpin risk assessments for TFA exposure in drinking water supplies and provide a robust evidence base to guide future regulatory standards and local policy decisions.

3.28.P-We315 Trifluoroacetic Acid (TFA) in the Environment: Multiple Sources and Regulatory Challenges

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Trifluoroacetic acid (TFA) is an extremely persistent and highly mobile fluorinated compound, increasingly detected in aquatic environments, soils, and biota worldwide. As the smallest short-chain perfluoroalkyl carboxylic acid, TFA combines a $-\text{CF}_3$ group with a carboxyl function, conferring complete water miscibility and exceptional chemical stability. These properties result in resistance to natural degradation and conventional water treatment, leading to accumulation in the water cycle and wide-scale dispersion.

TFA originates both from direct emissions during fluorochemical production and from the degradation of numerous precursors, including hydrofluorocarbon refrigerants such as R1234yf, blowing agents, plant protection products, pharmaceuticals, and biocides. Key entry pathways include atmospheric deposition, agricultural leaching, industrial discharges, and wastewater treatment. Over the years, TFA has become the most widespread water contaminant and one the main sources is due to PFAS pesticides, the use of which involves

deliberate dispersion in agricultural fields. EFSA has identified at least 56 PFAS pesticides under the ongoing peer review process and 12 PFAS active substances shown to produce TFA in soil and/or groundwater. Other anthropogenic sources remain poorly quantified, complicating source attribution and environmental modelling. Emission data are fragment and vary across regions and sectors.

Monitoring across Europe indicates that TFA is widely detectable in surface waters, groundwater, soils, crops, and food products, suggesting progressive environmental transfer. Although toxicological effects occur only at concentrations far above current environmental levels, the combination of extreme persistence, mobility, and ongoing precursor use implies continued growth of environmental loads in the coming decades. This raises potential conflicts between drinking water production, agricultural practices, and ecosystem protection. Regulatory measures are currently fragmented and lack harmonized limits. Evaluation processes in different regulatory areas can lead to different conclusions regarding the use of PFAS. European initiatives, including proposed PFAS restrictions under REACH (Reg. EC 1907/2006), aim to reduce inputs, yet coverage of major TFA precursors remains uncertain.

Disclaimer/Disclosure:

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3.28.P-We316 Policy Analysis for the Identification and Quantification of European Sources of Trifluoroacetic acid

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Trifluoroacetic acid (TFA) is very mobile, contaminates drinking water, is difficult to remediate, and is reprotoxic to humans (category 1B). It is also an anthropogenic pollutant, widespread throughout the environment, whose concentration is increasing. Understanding the relative size of different TFA sources to the environment is important. This will help assess the effectiveness of current regulatory efforts in reducing TFA emissions and inform targeted future regulatory efforts. This policy analysis seeks to complement existing environmental monitoring campaigns by determining the annual volumes of TFA and TFA precursors being placed on the European market.

TFA precursor volumes were determined using import, export, manufacturing, and sales data from the European Economic Area (EEA). The amount of TFA emitted to the environment was calculated for different uses: TFA as a basic chemical, PFAS (per- and polyfluoroalkyl substances) uses covered by the European Union's proposed REACH restriction (divided into fluoropolymers, fluorinated gases, PFAA and PFAA precursors), human medicinal products, veterinary medicinal products, and plant protection products. This data was combined with molecular mass, number of C-CF₃ groups, TFA molar yield, and emission factors. Where data was available, substance specific calculations were performed, otherwise grouped values were used. Uncertainty was quantified through a bounding analysis, so as to generate a best-case, and worst-case scenarios.

The results show the annual input of TFA to the environment from different sources in the EEA. For certain uses, country specific calculations are also included for comparison (Germany, Netherlands, and Switzerland). The largest TFA sources from the EEA are fluorinated gases (REACH uses) and plant protection products. Although it was the smallest source investigated, this study is also the first one to quantify TFA emissions from veterinary medicinal products, filling a data gap previously identified by European regulators.

This study supports the hypothesis that the REACH restriction should be effective in reducing TFA emissions to the environment (if implemented in its current format). However, this alone would be insufficient to eliminate emissions to the environment, as other sources (such as plant protection products) lie outside the scope of the REACH restriction. If TFA emissions to the environment are to be minimised, further regulatory action should be taken.

3.28.P-We317 Derivation of Guidance Values Under the EU Drinking Water Directive: the Case Study of Trifluoroacetic Acid

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The definition of a 'relevant metabolite' in water intended for human consumption (as per Drinking Water Directive) is based on risk, i.e. the function of hazard and exposure, whereas the definition of a 'relevant metabolite' in groundwater is based on human health hazard alone ("has certain toxicological properties that are considered unacceptable") in Article 3(32) of the Plant Protection Product Regulation, which is also applicable in the context of the Ground Water Directive. Therefore, a metabolite of a pesticide that is considered relevant under the PPP Regulation and under the GWD, is not necessarily also a relevant metabolite under the DWD, and the DWD does not include any guidance value for substances that are identical with drinking water relevant metabolites of PPPs but that are present in the drinking water due to such other uses and/or sources. As a consequence, different Member States may define different guidance values for a given substance.

In order to explore this topic, the case study of trifluoroacetic acid is considered. TFA is a metabolite of several active substances in PPPs that contain a C-CF₃ moiety and is also the terminal degradation product of a range of commercially used substances, such as next-generation refrigerants and propellants in spray cans. Since an EU-harmonised concept for the derivation of drinking water guidance values for TFA is unavailable, various guidance values have been proposed or implemented on the Member State level for drinking water which may cause uncertainty with respect to the adequacy of such values. As such, the scientific rationale underlying health-based guidance values for TFA in drinking water that have been set or proposed on the Member State level are reviewed in this study.

Finally, a science-based approach for deriving guidance values for substances in drinking water is proposed. Application of this approach to TFA as an example yields a health-based guidance value of 294 µg/L TFA in drinking water, and a supplementary guidance value of 56 µg/L to cover specific vulnerable population groups. Scientists from relevant stakeholders like executive and regulatory bodies, academia, water supply companies and industry are encouraged to align on an EU-harmonised approach to derive scientifically justified and robust guidance values for substances in drinking water to adequately protect consumers.

3.28.P-We318 Structural Impact on the Aquatic Fate of Aryl-CF₃ Contaminants: Persistence, Defluorination, and Formation of TFA

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The substitution of fluorine atoms in organic molecules results in advantageous properties for applications in industrial, household, health and agricultural applications. However, once in the environment, these compounds, many of which are considered per- and polyfluoroalkyl substances (PFAS), can be persistent and cause harmful effects. In the pharmaceutical and agrochemical industries, fluorine atoms are added either directly to an aromatic ring as a single fluorine (aryl-F) or as trifluoromethyl groups (aryl-CF₃). Our interests in aryl-CF₃ chemicals in the environment are threefold. First, these chemicals are of interest because they are designed to be biologically active and thus may have unintended biological impacts if they persist in aquatic ecosystems. Second, some aryl-CF₃ can form trifluoroacetic acid (TFA) in the environment, which is considered the smallest PFAS. Sources of TFA are poorly constrained and environmental concentrations continue to grow. The contribution of aryl-CF₃ to environmental TFA concentrations is unknown. Third, complete mineralization of anthropogenic organofluorine compounds is the goal after desired usage. As many aryl-CF₃ undergo defluorination in the environment, we aim to understand why certain structures undergo more defluorination than others to help inform the design of greener chemicals. This presentation will report our results of kinetics and products of hydrolysis and aquatic photochemical reactions of a suite of aryl-CF₃ to understand how their structure impacts persistence, defluorination, and formation of TFA. Some aryl-CF₃ form up to 10% TFA due to photochemistry, while the remainder of the aryl-CF₃ undergoes defluorination to fluoride. Conversely, some undergo full defluorination and do not form TFA, while others are stable at the C-F bond. Our use of ¹³C isotopic labels and high-resolution mass spectrometry allowed for the tracking of sequential oxidations to the aromatic ring resulting in ring cleavage to form TFA.

3.28.P-We319 Atmospheric Deposition of TFA from Urban, Rural, and Remote Regions

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The dominant fate of atmospheric trifluoroacetic acid (TFA) is wet and dry deposition. Comprehensive monitoring of this deposition is needed to understand its spatial and temporal variation. We have deployed custom atmospheric deposition samplers in various locations in Canada (e.g., urban Toronto, rural Ontario) to characterize spatial variability in a region. We have also deployed the samplers in Georgetown, Guyana, an area in which pollutants are rarely reported. All samplers were deployed for at least one year with samples collected approximately monthly. Samples were analyzed using ion chromatography coupled to mass spectrometry. Concentrations and fluxes in atmospheric deposition will be presented, as well as the implications for TFA sources and fate.

3.28.P-We320 Bioaccumulation and Toxicity of Trifluoroacetic Acid (TFA): Weight-of-Evidence for Environmental Risk Assessment

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Trifluoroacetic acid (TFA) is an ultrashort-chain fluorinated acid increasingly detected in rainwater, surface waters and groundwater worldwide. Its extreme persistence, high mobility and rising environmental concentrations have prompted regulatory interest, yet its behavior in aquatic organisms remains less well characterized than other per- and polyfluoroalkyl substances (PFAS). A clearer understanding of its bioaccumulation potential and aquatic toxicity is needed to support environmental risk assessment and the development of scientifically grounded water-quality benchmarks and standards.

The purpose of this work was to compile and evaluate available information on bioaccumulation and aquatic toxicity for TFA, and to integrate these data into a weight-of-evidence evaluation relevant to regulatory frameworks. A systematic literature review was performed to identify measured bioaccumulation factors and bioconcentration factors for fish and shellfish, as well as acute and chronic toxicity endpoints for algae, invertebrates and fish across freshwater and marine systems. All studies meeting minimum quality standards were compiled into a harmonized dataset for cross-species comparison and interpretation.

The available evidence indicates that TFA exhibits negligible bioaccumulation in aquatic organisms, consistent with its ultrashort-chain structure, and rapid elimination kinetics. The one published study of bioaccumulation in the aquatic macrophyte *Lemna minor* reported bioconcentration factors ranging from 1 to 1.6 indicating essentially no bioaccumulation. Aquatic toxicity studies across algal, invertebrate, and fish species show low sensitivity to TFA, with effect concentrations typically reported in the mg/L range.

Although the overall dataset supports a low hazard profile relative to other PFAS, notable uncertainties remain. Chronic toxicity data are limited, analytical confirmation is inconsistent in older studies, and marine taxa are underrepresented. The weight-of-evidence evaluation highlights the need for standardized chronic testing with analytical verification, updated fish bioaccumulation studies using modern methodologies, and expanded datasets across additional species and exposure conditions.

3.28.P-We321 Aerosol Microdroplet Photochemistry Enables Unexpected Transformations of Polyfluorinated Alkyl Contaminants that Produce Ultra-Short PFAS

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Transformations of PFAS under treatment or environmental conditions often result in shorter-chain perfluorinated alkyl chains. The high surface activity of PFAS allows their concentration to be enhanced through aerosolization in both a water treatment and sea spray aerosol context. This places the contaminants in a unique chemical environment at the air–water interface that can accelerate degradation kinetics and alter the transformation reaction pathways. We explored this using an oxidation flow reactor that exposed PFAS-containing aerosol droplets to 185 or 254 nm UV radiation. This generates gas-phase ozone as well as gas and aqueous-phase hydroxyl radicals relevant to both water treatment and tropospheric chemistry without requiring addition of chemical reagents. We compared substrate degradation rate and extent, defluorination, and transformation products between aerosol microdroplets and bulk using both targeted and nontargeted high-resolution mass spectrometry. Chemical ionization mass spectrometry using iodide as the reagent ion enabled real-time observations of a series of small volatile fluorinated reaction products not captured by LC-MS analysis. We observed fluoride production and a homologous series of perfluorocarboxylic acid (PFCA) transformation products, indicating perfluoroyl chain-shortening of the 6:2 FTCA in addition to removal of the –CH₂C(O)OH head group. Aerosol microdroplets produced much faster oxidation kinetics and deeper degrees of transformation and defluorination, all the way down to TFA from a C₆ perfluoro alkyl precursor. The reactants were produced by VUV photolysis of O₂ and H₂O using 185 nm, or of hydrogen peroxide under 254 nm illumination relevant to cloud water chemistry.

These findings could provide a lower-cost PFAS remediation and mineralization approach, which can enable water treatment and reuse by removing ultrapersistent micropollutants. We will present recent work evaluating the transformations of ultra-short PFAS such as TFA, where treatment of PFAS often gets stuck and become a source of ultra-short PFAS. These findings also imply that PFAS may experience unexpected transformations in atmospheric aerosol driven by photo-generated hydroxyl radical and interfacial chemistry. This may represent an additional secondary source of ultrashort PFAS such as TFA which is routinely measured in the troposphere and surface waters.

3.28.P-We322 Analytical Development for the Qualification and Quantification of Environmentally Relevant Concentrations of Trifluoroacetic Acid (TFA)

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Trifluoroacetic acid (TFA) is an environmental contaminant of increasing focus, arising as a terminal degradation product of fluorinated substances. Its ubiquity and chemical persistence present analytical challenges, particularly when quantifying environmentally relevant concentrations during sample analysis from simulation studies. This work presents the development and validation of a robust analytical framework for the qualification and quantification of TFA across multiple environmental matrices.

Method development focused on differentiation of sample-derived TFA from background residues through the use of ¹³C-labelled TFA as a stable isotope reference standard. Quantitative performance was assessed using a quantitative triple quadrupole mass spectrometer, while complementary qualitative assessments of method selectivity and molecular confirmation were conducted on a Thermo Eclipse Orbitrap high-resolution mass spectrometer. Sensitivity studies demonstrated sub-nanogram per litre detection limits, with reproducible quantification achieved across complex matrices following tailored sample preparation and ion suppression control strategies.

The analytical methods enable confident identification and quantification of substance derived TFA at environmentally relevant concentrations, addressing a key analytical barrier in assessing its occurrence, persistence, and potential ecological impact. This work contributes to improving trace-level detection to support environmental risk assessments of organic compounds containing trifluoromethyl functional groups which have the potential to generate TFA as a terminal metabolite.

Track 4: Ecological and Human Health Risk Assessment of Chemicals, Mixtures and Stressors and Risk Mitigation Strategies

4.01.A Advancing the Environmental Risk Assessment and Management of Cosmetics, Sunscreens and Personal Care Products

4.01.A.T-01 Combining SCCS And BPR Product Type 1 Exposure Frameworks To Derive Consumer Based Environmental Risk Assessment For Cosmetic Ingredients

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Cosmetic products can contribute to emissions to wastewater during normal use as they are disposed down-the-drain. However, environmental exposures are regulated via REACH and not the Cosmetic Product Regulation. Hence, no dedicated environmental exposure approach exists for cosmetics. We propose to use a combination of two approaches which are both well-grounded in existing regulations but in their combination new to cosmetics. We use the Scientific Committee on Consumer Safety (SCCS) Notes of Guidance for input calculation assuming all cosmetics are washed-off. For exposure, we use the Biocidal Products Regulation (BPR) Product Type 1 (PT 1) guidance which also is a down-the-drain scenario established for estimating releases from human hygiene products to wastewater. We adapt the structured exposure logic developed under the BPR, where environmental risk assessment is used to demonstrate that uses do not cause “unacceptable effects” on the environment, to build a transparent emission basis for cosmetic ingredients.

Our method integrates SCCS default parameters with PT 1 emission equations. For each category, SCCS values for amount per use are combined with ingredient concentrations to calculate daily emissions to wastewater. The method quantifies the emissions entering wastewater during consumer use. These emissions serve as inputs in EUSES, where they are converted into Predicted Environmental Concentrations for surface waters (PEC –

surface water).

Case examples using typical cosmetic ingredients (e.g. UV-filters, Preservatives) illustrate how the method captures differences in product occurrence and consumer use patterns, enabling more realistic and transparent emission estimates.

The combined SCCS–PT 1 framework provides a reproducible, well-documented and regulation-consistent basis for estimating emissions of cosmetic ingredients, helping to close a key gap in cosmetic environmental assessment.

4.01.A.T-02 Commercial Organic UV filters in Sunscreens and their Ability to form Novel Disinfection By-products

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Organic UV filters and their toxicity have been the focus of research in recent years. However, their degradation and transformation products remain largely undefined. Here we show a systematic non-targeted approach to discover disinfection by-products (DBPs) that are produced upon chlorination of UV filters with free chlorine at different doses. Non-targeted ultrahigh resolution 12 Tesla Fourier transform Ion Cyclotron Resonance Mass Spectrometry (FT ICR MS) was used to discover novel DBPs arising from commercial organic UV filters in sunscreens that also have been found in reasonably high concentrations in the aquatic environment where people are bathing. As expected, the susceptibility of direct chlorination of these aromatic organic compounds depends on the activation of the aromatic ring structure. However, diverse DBPs were discovered which raise concerns in creating high molecular weight DBPs of currently unknown toxicity. These DBPs have the potential to be abundant in chlorinated outdoor swimming pools, as well as in treated and disinfected wastewater. For example, avobenzone seemed relatively inert to chlorination, but octocrylene showed the consistent production of 7 abundant DBPs containing up to three chlorine atoms in negative mode electrospray ionization. DBPs formed from homosalate, octisalate and OD-PABA were also evaluated. The obtained data confirmed that rather diverse DBPs form from these compounds upon chlorination and that further evaluations in real word scenarios are needed to address this potential risk and exposure. The persistence of these DBPs are currently unknown, but all these DBPs accumulated over chlorination time and dose, suggesting that these are end-products and not intermediate compounds.

Disclaimer/Disclosure:

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4.01.A.T-03 How time and place define the concentration of UV filters off a recreational beach in Florida, USA

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Ultraviolet (UV) filters are a diverse group of organic and inorganic chemicals that have a wide range of commercial applications, including as sunscreen active ingredients, and have been found in coastal locations reflecting the extent of recreational activity. Despite the potential for recreational source inputs to vary over the day, typically monitoring programs only analyze one or a few discrete samples and do not capture this temporal variation. To investigate this, 3 composite samples were collected along 2 100m transect locations at different distances from the shore (T1; nearshore in the bathing zone and T2 ~50m from the shoreline) at multiple times over 24-hours. Samples were immediately processed by filtering (GFF) and solid phase extraction to prepare the 'dissolved fractions'. The GFF filters were frozen and later extracted via hexane to prepare the 'particulate' fractions. All extracts were analyzed quantitatively for 12 organic UV filters using LC-MS/MS.

Oxybenzone (BP-3), octocrylene (OC), homosalate, octisalate, avobenzone, octinoxate, and, OD-PABA were measured in all samples, with higher levels in May, reflecting higher numbers of recreational users. Concentrations were higher at T1 and reduced significantly at T2. All UV filters and the septic-tracer caffeine

followed similar temporal trends with peak concentrations detected in the mid afternoon, reflecting recreational use. This dataset allows for the calculation of time weighted average concentrations (TWAs). Higher concentrations of OC were observed in the 'particulate' phase reflective of its lower solubility and higher logKow, whereas, BP-3 concentrations were nearly all in the 'dissolved' phase matching their physio-chemical properties. Concentrations of the lower solubility UV filters were not as different between March and May as the more soluble BP-3 concentrations were suggesting concentration saturation.

Overall, these results indicate that aquatic organisms in popular recreational areas may encounter UV filter concentrations that are highly variable over a 24-hour period rather than a steady environmental exposure, and that concentrations rapidly decline in the bathing zone after people leave. These results highlight that both location and time is important in environmental risk assessments for UV filters. Furthermore, to understand total concentrations and exposure routes it is important to conduct assessment of partitioning by filtering samples.

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4.01.A.T-04 Spatial and Seasonal Variations in Concentrations of UV Filters and Preservatives Across Yorkshire's Rivers Revealed by Continuous River Monitoring

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Ultraviolet (UV) filters and preservatives are widely used in personal care products. Their extensive use and continuous release into aquatic systems have led to widespread occurrence and raised concerns about environmental impacts. Although many studies have investigated their occurrence, few have achieved the temporal resolution needed to capture seasonal variations or identify key drivers of concentration differences. To address this gap, we conducted a one-year, high-resolution monitoring campaign across 19 sites in nine river catchments in Yorkshire, United Kingdom, spanning land uses from densely populated urban areas to rural arable and livestock regions. Sampling was performed every six hours using automatic samplers, and composite samples were prepared every four weeks. Thirteen target chemicals were selected for monitoring, and their concentrations were analysed using liquid chromatography–tandem mass spectrometry or gas chromatography–mass spectrometry. Ultraviolet filters were detected more frequently than preservatives, with Ensulizole showing the highest detection frequency and mean concentration. Preservatives such as parabens and phenoxyethanol were present at lower levels. Significant differences were observed among land-use categories and seasons for both chemical classes. For UV filters, peri-urban catchments exhibited the highest flow-corrected fluxes, exceeding those in arable, livestock, and urban areas. Preservative fluxes were higher in city, peri-urban, and market-town catchments than in agricultural areas. Both patterns reflect the impact of mixed residential and light-industrial activities through domestic wastewater, urban effluents, and surface runoff associated with personal care product usage. Significant seasonal variation was also observed. UV filter fluxes peaked in spring, exceeding other seasons, whereas preservative fluxes were highest in summer. These patterns indicate that elevated fluxes during warmer months reflect increased product consumption and enhanced releases via domestic wastewater and urban effluents. The strong temporal coupling at certain sites further suggests overlapping emission pathways and shared hydrological controls. These findings provide insights into the behavior and sources of personal care product-related contaminants, supporting improved regional water quality management and regulatory strategies.

4.01.A.T-05 Application of Probabilistic Emissions Modelling to Estimate UV Filter Concentrations at a Recreational Beach in Florida

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UV filters (UVFs), the active ingredients in sunscreen products, are subject to scrutiny from the scientific and regulatory community due to their potential risk to organisms in aquatic compartments. To assess this risk,

environmental exposures are best characterized by combining exposure modelling with well-designed monitoring studies. While properly conducted monitoring studies provide the most definitive exposure data, they are snapshots of individual scenarios in time and space. Modelling has the potential to assist in extrapolating monitoring data to additional sites with similar visitation patterns and hydrology. To support UVF risk assessment, a probabilistic model incorporating environmental fate processes and consumer habits and practices was developed to represent the recreational beach exposure pathway and predict the UVF emissions due to direct release of skin-applied sunscreen products to aquatic environments. A joint monitoring and modelling study of UVF concentrations at Calusa Beach in Bahia Honda State Park in the Florida Keys was conducted to elucidate spatial and temporal UVF concentration trends and to provide a case study to evaluate the predictive ability of the probabilistic UVF emissions model. Measured UVF concentrations were highest in the afternoon, when the number of swimmers/bathers peaked. Subsequently, as recreational activities declined, UVF concentrations significantly decreased due to dilution/dispersion. Model performance was represented by the ratios of the predicted median values (describing the log-normal distributions of modelled concentrations) to the average concentrations measured in triplicate water samples. The UVF emissions model performed well (i.e., within a factor of 4) at predicting measured concentrations for the five UVFs during peak recreational activity at two sampling sites. On average, the model overpredicted the measured values by a factor of 1.8. The reasonable agreement between the predicted and measured values indicates the probabilistic emissions model can be a useful tool for characterizing aquatic UVF exposure at recreational beaches.

4.01.B Advancing the Environmental Risk Assessment and Management of Cosmetics, Sunscreens and Personal Care Products

4.01.B.T-01 Integrated Approaches to Assess Bioavailability and Bioaccumulation of Water Soluble Polymers

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Water soluble polymers possess structural and physicochemical features that inherently limit biological uptake, yet current regulatory expectations increasingly call for clear and transparent demonstration of their bioavailability and bioaccumulation behavior. Their large molecular size, extensive hydration, low lipid affinity, and limited membrane permeability contribute to minimal internal exposure across human and environmental pathways.

This presentation outlines a weight of evidence framework that integrates mechanistic principles of bioaccumulation, toxicokinetic information from mammalian and environmental systems, and emerging analytical approaches to assess the bioavailability and bioaccumulation potential of water soluble polymers. The approach considers attributes such as molecular size, hydrodynamic diameter, solubility behavior and links these properties to expected uptake constraints. Empirical indicators and toxicokinetic observations are used to demonstrate the consistently low internal exposure observed for representative examples of water soluble polymers, reinforcing their low potential for bioaccumulation. New methods and approaches including solvent partitioning, and approaches to simplify testing compared to expensive fish studies (eg sharpening toxicokinetic studies with CaCo2 assays) will be shared.

Together, these elements provide a coherent and scientifically grounded basis for evaluating water soluble polymers. Importantly, many of these approaches are referenced in existing regulatory approaches around the world- albeit not all the same. However, this link and references do create an ability to link the mechanistic, and new analytical approaches within a modern regulatory context. We propose a framework and toolkit which illustrates how mechanistic understanding and empirical evidence can be combined to assess bioaccumulation potential in a manner that reflects the unique properties of these materials and supports transparent, science based assessments.

4.01.B.T-02 Risks of Mixtures of UV Filters to Freshwater Algal and Cyanobacterial Species

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UV filters (UVFs) are extensively used in personal care products and have been increasingly detected in aquatic environments worldwide. Although the toxic effects of individual UV filters on standard aquatic

ecotoxicological test species have been studied, the understanding of their effects on species more widely, as well as their combined effects, remains limited. This study explored the risks of five commonly detected UVFs, homosalate, octocrylene, benzophenone-3, ensulizole, and avobenzone, to freshwater algal and cyanobacterial species. Laboratory bioassays were conducted using twelve phytoplankton and periphyton species (five chlorophytes, two cyanobacteria, and five diatoms) using single UVFs and mixtures of UVFs with growth inhibition employed as the toxicity endpoint. Bioassay results were used to evaluate the concentration addition (CA) and independent action (IA) mixture toxicity models for predicting mixture effects. The models were then used, alongside an extensive monitoring dataset for UVFs in Yorkshire's rivers, to establish the potential risks of these chemicals to freshwater systems. Results from single-compound tests demonstrated pronounced interspecific and higher taxonomic rank variability in sensitivity, with diatoms generally exhibiting higher susceptibility to UVF exposure than chlorophytes and cyanobacteria. Among the tested species, the diatom *Aulacoseira granulata* was identified as the most sensitive. Model evaluation using the model deviation ratio (MDR) indicated that the CA model provided consistently more accurate predictions of observed mixture toxicity compared to the IA model indicating that the toxicological interaction of the tested UVFs is predominantly additive. Use of the CA model alongside UVF monitoring data yielded mixture risk quotient values below 1, indicating that the selected UV filters currently pose a low risk to aquatic environments in the study region under typical exposure conditions.

4.01.B.T-03 Aquatic Risk of Fragrance Materials: Advancing Prioritization in Aquatic Systems

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For more than 50 years, the Research Institute for Fragrance Material (RIFM) has led programs to demonstrate the human and environmental safety of more than 3,000 fragrance materials manufactured and used by their members. The Environmental Program sponsors testing and academic research on the fate and effects of fragrance materials in aquatic compartment. In addition, RIFM conducts environmental risk assessments and screens fragrance materials using the RIFM Environmental Framework published almost 20 years ago (Salvito et al. 2002. Environ. Toxicol. Chem., 21: 1301-1308). While the Framework has proved to be an effective tool, its scope was limited to the United States and Europe. RIFM recognized the need to expand the geographic scope of those assessments to include regions with rapidly expanding usage of fragrance materials. Also, advances in environmental exposure science and ecological hazard characterization over the past two decades offer an opportunity for the Framework to showcase cutting edge science and data analytics. This presentation will highlight recent advances of the Framework that feature expanded geographies, real-time access to the most current population and hydrological data, mechanism of action material categorization and the ability to reassess the entire inventory of fragrance materials nearly instantaneously at the push of a button.

4.01.B.T-04 Advancing Environmental NGRA: Transferring a 10-Step Read-Across Framework for Cosmetic Ingredients

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Next-Generation Risk Assessment (NGRA) provides a structured, mechanistic, and non-animal approach for chemical safety evaluations. While NGRA concepts are increasingly established in human safety assessment, their application to environmental safety remains at an early stage. This work demonstrates how a 10-step NGRA framework, originally developed for human safety evaluations of cosmetic ingredients, can be systematically adapted for environmental risk assessment.

Using triclosan as a data-rich model compound, we assessed how the framework performs when environmental exposure, fate, and hazard information are integrated with New Approach Methodologies (NAMs). The adapted workflow covers scenario definition and data curation (Tier 0), bioavailability modelling and mode-of-action hypothesis building (Tier 1), and the derivation of environmental points of departure from NAM datasets (Tier 2). Mechanistic information from transcriptomics, high-throughput bioactivity assays, and metabolic and endocrine-related pathways was combined with QIVIVE and bioaccumulation models to identify sensitive biological targets and derive NAM-based PNEC values, which subsequently allow the calculation of a risk quotient and thereby a complete environmental risk assessment.

Applying the framework to triclosan resulted in NAM-derived PNECs that align with—and in part are more protective than—existing regulatory PNECs. The case study confirms that environmental NGRA can generate transparent and biologically meaningful evidence for risk characterisation without new vertebrate testing.

This work illustrates the feasibility of translating a human-safety NGRA framework to environmental safety and highlights its potential for broader implementation across sectors. Strengthening data integration, mechanistic interpretation, and cross-species extrapolation will be key steps towards establishing NGRA as a future-ready paradigm for environmental protection.

4.01.B.T-05 Cosmetics Claims: Need for Enhanced Risk Communication on Cosmetic Ingredients

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Cosmetic products are a notable source of chemical exposure in daily life, as consumers use multiple products daily (e.g., shampoo, toothpaste, deodorant, moisturiser), each containing complex mixtures of ingredients. Cosmetics exposure is directly through skin contact, and indirectly via inhalation of volatile components, eye and oral routes. Despite extensive exposure, information available to the public about cosmetic ingredients, their potential hazard for human health and real-world risk is fragmented, highly technical and uneven in quality. The EU's regulatory database and opinions are publicly accessible but are written for experts. Smartphone apps have emerged as an easy way for consumers to access information, but it is unclear what data is used, the basis for their scoring and whether they differentiate between hazard and risk.

To gain a better understanding of the effectiveness or otherwise of these "scoring" approaches for risk communication to consumers, a systematic analysis of the apps available in English and their scoring systems was performed, supplemented by an online survey of general public opinion on the information available regarding ingredients in cosmetics and what drives their purchasing choices.

A structured search of Smartphone apps was performed in Nov. 2025 on Google Play/Apple App Store to aggregate all consumer facing cosmetics scoring apps into one dataset. The review used the PRISMA workflow, predefined keyword combinations and inclusion/exclusion criteria, identifying >150 mobile apps that display ingredient- or product-level scores to users in a standardised format (numeric, colour code, icons or labels). The survey of consumer perceptions was carried out May-Nov. 2025 and examined the key factors influencing purchasing decisions, the relevance of product claims and labels, and the role of digital tools in shaping choices. >300 responses were collected and results indicate that consumers reported moderate to very concerned regarding cosmetic product safety. Presence of controversial ingredients (e.g., parabens), absence of safety certifications or testing and negative social media or negative product reviews are among the factors that drive consumer perception of a cosmetic being potentially hazardous.

The results show a clear need for much stronger management of communication of risks from cosmetics / cosmetic ingredients to the public, ideally via establishment of an independent body to oversee such communication.

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4.01.P Advancing the Environmental Risk Assessment and Management of Cosmetics, Sunscreens and Personal Care Products

4.01.P-Mo272 Spatio-Temporal Patterns of UV Filters in the Baltic Sea and Their Distribution at Different Depths

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The occurrence of organic ultraviolet (UV) filters is of increasing scientific concern due to their potential as endocrine disruptors and their ability to bioaccumulate 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, with its unique topography and freshwater input that carries pollutants from human activities in the catchment area, is recognized as one of the most heavily impacted regional seas in terms of chemical pollution. One group of these pollutants is UV filters, which primarily enter the Baltic Sea 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, particularly in the surface waters around the Warnow Estuary and other coastal sites. The main objective of this study is to determine if increasing levels of UV filters in the coastal Baltic Sea are transported into offshore waters and to identify the areas with the highest levels of pollution. Water sampling conducted during the spring-summer and autumn-winter seasons of 2023

and 2024 were used to evaluate the seasonal variability of UV filter concentrations across the Baltic Sea. This study also provides a comprehensive comparison between the distributions in the coastal areas and the open sea, while considering variations across different depths in the open waters. Among the UV filters detected, octocrylene (OC), PBSA, and UV-P were the most prevalent. The highest concentrations of OC were found in water samples from coastal areas during the summer, reaching as high as 162.6 ngL⁻¹. The peak concentration of PBSA, at 732.8 ngL⁻¹, was recorded in the coastal area in November, while the highest concentration of UV-P, measured at 34.3 ngL⁻¹, was found in the open sea during the same month. Other UV filters, such as BP-4, were detected at notable concentrations in coastal areas. No significant differences in UV filter concentration distributions across different depths were observed, indicating a uniform distribution of UV filters throughout the water column in the open sea of the Baltic Sea. In conclusion, UV filters were found in both the open sea and coastal regions of the Baltic Sea throughout the year. These findings provide clear evidence of the widespread occurrence and distribution of UV filters in the Baltic Sea.

4.01.P-Mo273 Environmental occurrence of octocrylene in marine and freshwater systems: A critical review

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The use of octocrylene has come under regulatory scrutiny, with concerns regarding its potential environmental risk. A main challenge in assessing environmental risk relates to uncertainties associated with exposure quantification, specifically with the use and release of octocrylene into marine and freshwater systems. A systematic and critical literature review is conducted in this study, consistent with the Criteria for Reporting and Evaluating Exposure Data (CREED), with the primary aim to collate measured environmental concentrations (MECs) of octocrylene in surface water systems. The results of the study, consequently, include considerations regarding the reliability and usefulness of the available data, to quantify and/or represent reasonable approximations of environmentally relevant exposure to octocrylene. Significant procedural and methodological inconsistencies were involved among the available studies regarding surface water and sediment sample collection, processing, and analyses. Therefore, accompanying discussion in this study draws attention to the need for well-defined monitoring studies capable of acquiring concentration data that are representative of environmental systems and the need to either increase the number and frequency of samples collected or to consider the adoption of time-weighted passive sampling methods at sites to account for spatial, diurnal, and seasonal variabilities. Nonetheless, the existing data provides some understanding of central tendencies in MECs, which may be useful in ground truthing ongoing regulatory environmental risk assessments. The geometric mean concentrations for octocrylene in surface water reported at sites associated with recreational activities are similar in freshwater (30 ng/L) and marine (37 ng/L) systems. Similarly, the geometric mean concentrations of octocrylene in sediments influenced by potential sources of octocrylene are also relatively consistent for samples collected from both estuarine (6.9 ng/g dw) and freshwater riverine (4.3 ng/g dw) systems.

4.01.P-Mo274 ChemRadar: Detecting Chemical Hazards on the Horizon

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ChemRadar is a forward-looking initiative aimed at improving the detection and prioritization of emerging chemical threats in the drinking water sector. Traditional monitoring systems are often reactive, focusing on known contaminants, which leaves utilities vulnerable to unforeseen risks. ChemRadar addresses this gap by introducing a structured workflow that integrates existing tools and data sources into a flexible, adaptive system.

The workflow is organized into three analytical stages: screening, signaling, and prioritization. Screening involves collecting broad information on chemical occurrence and emissions, even if data is incomplete or noisy. Signaling interprets patterns and trends to identify potential threats, while prioritization ranks substances based on factors such as exposure relevance, toxicity, persistence, and mobility. This non-linear approach allows users to start at any stage depending on their context and needs.

ChemRadar recognizes four distinct user domains—scientific, industrial, societal, and policy—each with unique perspectives and requirements. For example, scientists may focus on analytical methods and data gaps, while policymakers prioritize regulatory trends. Practical applications range from laboratory analysts detecting unknown contaminants to strategic managers planning long-term water safety measures.

The framework leverages a wide array of resources, including scientific databases, regulatory platforms, industrial data, and societal tools. It also incorporates computational models and text-mining techniques to enhance foresight. Two complementary implementation strategies are proposed: a top-down approach, which scans broad chemical space for preventive insights, and a bottom-up approach, which focuses on contaminants detected in drinking water for immediate relevance. Combining these strategies improves predictive power and reduces blind spots.

In the next phase, we are evaluating the system for a series of potential threats, ranging from individual chemical groups to more generic trends in uses /applications of groups of chemicals to generic trends and changing activities that can lead to emission of chemicals. These cases are used evaluate the value and applicability of the ChemRadar concept/workflow in collecting information, identifying knowledge needs and gaps and supporting the evaluation of the risk for the drinking water sector. Preliminary results of a selection of these cases will be presented

4.01.P-Mo275 Filling Data Gaps in Chemical Risk Assessment: Multi-Trophic Evaluation of Cinchonidine and Its Production Byproducts

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Cinchonidine is a quinoline alkaloid and a common chiral synthetic intermediate in pharmaceutical synthesis, which can enter wastewater through laboratory use, drug manufacturing, or disposal of reaction residues, potentially exposing aquatic organisms to this compound. Although structurally related to regulated antimalarial compounds increasingly used in drug production, ecotoxicological data for cinchonidine remain extremely limited. The toxicity assessment of waste generated during the production of the active pharmaceutical ingredient (API) is a key strategy for advancing green pharmaceutical manufacturing. In this study, pure cinchonidine and cinchonidine-containing waste residues generated at different stages of API production, supplied by a pharmaceutical company (AstraZeneca), are being evaluated in aquatic model organisms: *Aliivibrio fischeri* (MicroTox), *Saccharomyces cerevisiae* (Yeast Estrogen Screen Assay), *Raphidocelis subcapitata* (OECD Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test), *Daphnia magna* (OECD Test No. 202: *Daphnia* sp. Acute Immobilisation Test), and *Danio rerio* (OECD Test No. 236: Fish Embryo Acute Toxicity (FET) Test). Results from this case study will help clarify the environmental hazards associated with cinchonidine-containing wastes and support the industry's greener pharmaceutical development, highlighting the need for environmental risk assessment approaches that explicitly consider the toxicity of production residues, such as cinchonidine. Moreover, this work highlights the importance of collaboration between academia and the pharmaceutical industry for the development of greener pharmaceuticals.

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4.01.P-Mo276 Chemical Contaminant Inhibition of Settlement and Survivorship in Caribbean Corals

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Early life stages of scleractinian corals are generally regarded as more sensitive to contaminants compared to adults. Spawning and larval development of most coral species on Florida's Coral Reef occurs through the summer and fall, which coincides with substantial rain-event driven events associated with risk of exposure to chemical contaminants in the water column and sediment/substrate. The inhibition of larval settlement and/or metamorphosis, which are crucial developmental steps and ultimately determine survival, have been examined for only a very limited range of chemical contaminants and coral species.

Montastraea cavernosa and *Pseudodiploria clivosa* larvae were exposed to contaminants as nominal dissolved concentrations or pre-contaminated settlement tiles, and tiles checked for settlement and metamorphosis daily

for 96 hours or until no observable swimming larvae are found (i.e. either settled or perished). Chemicals included metals (copper, arsenic, zinc), hydrocarbons (chrysene, fluoranthene, pyrene, phenanthrene), and common organic pollutants (tributyltin, diuron, and octocrylene) as these chemicals are waterborne and/or sediment-associated contaminants recognized for potential toxicity. These contaminants were identified in a recent metanalysis as contaminants of potential impact as they have been detected at concentrations which exceed both EPA water quality standards and State of Florida threshold and/or probable effects levels in waters and sediments across the southeast coast of Florida.

For *M. cavernosa*, significant reductions in settlement were observed for dissolved exposures to fluoranthene, phenanthrene, pyrene, diuron, octocrylene, and tributyltin and for settlement tiles contaminated with zinc, pyrene, and octocrylene. Non-significant dose-related reductions in settlement were also observed for other dissolved and contaminated tile exposures. The test methodology consistently met or exceeded the typical 20% laboratory settlement success rate for *M. cavernosa*.

This study has thus far identified preliminary thresholds of effect for common inorganic and organic chemical contaminants exposure for settlement in Atlantic corals, and provided a framework for the establishment of standardized toxicity test methods for coral early life stages which are a current need and essential for determining the environmental risk of chemical contaminants on coral reefs.

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4.01.P-Mo277 Polyvinyl Alcohol-induced Cytotoxic and Oxidative Responses across Tissues of the Blue Crab *Callinectes sapidus*

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Polyvinyl alcohols (PVAs) are synthetic water-soluble biopolymers produced from polyvinyl acetate and widely used in industrial, medical, and personal care applications, including packaging films, detergents, contact lenses, and cosmetics. Over 65,000 tonnes enter aquatic ecosystems annually, and their slow degradation under limited environmental conditions raises concerns about their effects on marine fauna.

This study investigated the effects of PVA exposure on the blue crab *Callinectes sapidus*, an alien species in the Mediterranean Sea. Three exposure concentrations (0.5, 5, and 25 mg L⁻¹) and a control were used, and crabs were exposed for 10 and 20 days. Haemolymph was collected with sterile syringes, and after euthanasia in ice water, muscle, gill, and hepatopancreas tissues were dissected for analysis. Oxidative stress was assessed through the activities of antioxidant enzymes: superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GPx) and lipid peroxidation levels. Cell viability in haemolymph and hepatopancreas was evaluated, while statistical differences among treatments were analysed using the Kruskal–Wallis test followed by Dunn's post hoc comparisons. The overall biochemical response was interpreted using the Threshold-based Integrated Biomarker Response (IBR-T) index.

Results revealed a greater sensitivity of hepatopancreas cells to PVA exposure compared to haemolymph cells. Prolonged exposure induced clear oxidative stress, with increased antioxidant enzyme activity and lipid peroxidation, particularly at higher concentrations. After 10 days, the IBR-T index rose with exposure level in all tissues, reaching its highest values in the higher PVA concentration group, indicating early activation of antioxidant and detoxification processes. After 20 days, IBR-T values remained elevated, especially in muscle and hepatopancreas, suggesting cumulative stress effects. Overall, the study demonstrated a concentration and time dependent biological response to PVA, highlighting significant physiological alterations at the highest exposure level. These findings contribute to a better understanding of PVA–organism interactions and emphasise the need for further research on their environmental implications.

4.01.P-Mo278 Freshwater Toxicity of Polymer-Surfactant Mixtures Across Trophic Levels

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The development of advanced materials, particularly for application in personal care products (PCP), is shifting due to growing environmental concerns. Continuous release into wastewater systems makes them prone to become pseudo-persistent pollutants. Current regulatory assessments focus on individual compounds, but commercial formulations are complex mixtures in which components interactions can unexpectedly affect freshwater life.

This study, based on an industry-academia partnership, aimed to investigate the ecotoxicological behavior of three quaternized hydroxyethyl cellulose polymers common in PCPs (SoftCAT™ SK-M, SK-MH, and SL-5) alone and in combination with three surfactants: Sodium Lauryl Ether Sulfate (SLE2S), Alkyl Amido Propyl Betaine (APB), and Tallow Ester Quat (EQ). The cellulose polymers were tested at pre-established HC₅ values (Hazardous Concentration for 5% of species) from previous research: 0.00354 mg/L (SK-M), 0.00127 mg/L (SK-MH), and 95.5 mg/L (SL-5). Surfactants were used at 33-fold higher concentrations to maintain the typical 1:33 polymer-to-surfactant ratio found in commercial formulations. Eight treatments were used per polymer in each assay, including a negative control, individual compounds (polymer at HC₅; surfactants at 33 × HC₅), and their binary mixtures. Toxicity assessment comprised a battery of assays with freshwater organisms covering different trophic levels: the producer *Lemna minor* (yield and growth), primary consumers *Brachionus calyciflorus* and *Daphnia magna* (mortality and reproduction), and the secondary consumer *Danio rerio* (mortality, malformations, heart rate, body length).

Toxicity profiles were polymer- and species-specific. Combined and individual exposure of *D. rerio* to SK-M and surfactants altered the cardiac function, with combined exposures producing different effects than single compounds. SK-MH altered cardiac function only in combination with APB, where the mixture response was different from that of the polymer and surfactant alone. No significant effects were detected for *L. minor*, *D. magna*, or *B. calyciflorus* for SK-M and SK-MH. On the other hand, SL-5 led to high mortality in *D. rerio* and pronounced growth inhibition in *L. minor*, with the strongest effects produced by SLE2S and APB (alone and combined with SL-5). These results show that interactions among PCP ingredients can markedly change toxicity, suggesting current single-compound testing frameworks may underestimate mixture-related risks.

Disclaimer/Disclosure:

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4.01.P-Mo279 Natural complex substances: An experimental & in silico combination approach to evaluate ecotoxicity

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Natural complex substances (NCSs) are botanical extracts of unknown or variable composition containing long chain substances that are very difficult or impossible to identify,

The recommended approach to perform an environmental aquatic hazard assessment for UVCBs is water accommodated fraction (WAF) toxicity assays. Such methodologies have been used for the fragrance industry's essential oil products (termed Type 1 or NCS1. However, WAF lacks mechanistic interpretability. Its application in hazard and risk assessment is therefore questioned.

Bicherel and Thomas (2021) published an in silico method for toxicity estimation for complex mixtures using an equivalent of the WAF methodology deemed applicable to non-polar narcotic mixtures such as essential oils (NCS1). This in silico method mimics WAF studies for nonpolar narcotic mixtures using chemical activity principles. It accurately predicts acute and chronic ecotoxicity lethal or effect loading rate. Nevertheless, until now, the method had not been used for toxicity estimation of more complex substances than NCS1, e.g. NCS2 substances containing large fractions of unidentified constituents.

In this study the NCS was separated into three fractions: 1) "volatile," 2) "non-volatile but potentially bioavailable," and 3) potentially "inert" and analytically define them. The whole substance (WS) and each fraction were tested as water accommodated fractions (WAFs) for acute and chronic ecotoxicity to algae and daphnids. Their toxicity was predicted using (a) an in silico method adapted for mixture toxicity testing and (b) the additivity Classification Labelling and Packaging/Global Harmonised System (CLP/GHS) method, based on analytically determined composition.

The study demonstrated the importance of combining in silico and experimental approaches, especially for

complex substances such as NCS2. The fractionation approach provided quality empirical toxicity study results and these are in agreement with the in silico predictions. Thus it can be concluded that the experimental data validate the results of the in silico prediction while the latter support and thermodynamically explain the empirical results from the studies. On the other hand, CLP does not predict toxicity in any of the studies. The implication of these conclusions will be discussed.

4.01.P-Mo280 In Silico Eco/Tox Prediction Using Triad of QSAR model, Toolbox read-across and expert knowledge. Application for skin sensitization

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Across the variety of industrial sectors, including pharmaceuticals, cosmetics and personal care products, food additives and their associated regulatory assessments, there is a need to develop robust and reliable methods to reduce or replace animal testing. It is generally recognised that no single alternative method will be able to provide a one-to-one replacement for assays based on more complex toxicological endpoints. Hence, information from a combination of techniques is required. The current work suggests a combination of Quantitative Structure-Activity Relationship (QSAR) predictions, read-across (RA) analogues approach and expert knowledge for providing mechanistically reliable predictions. The combined approach is called “QSAR Triad” and will be demonstrated for predicting skin sensitization potential of a case study chemical. The first pillar of the QSAR Triad includes QSAR prediction from the OASIS TIMES software which is justified by identification of analogues supporting the prediction and analogues supporting the simulated metabolic pathway responsible for the metabolic activation. In addition, the system allows to consider the toxicokinetics of adduct formation and to quantify the extent of damage that could be caused to macromolecules (i.e., to skin proteins). All supporting information is automatically organized and could be reported via the software according to the QSAR Assessment Framework (QAF) approach. The second pillar of the QSAR Triad relies on RA prediction obtained in QSAR Toolbox and is based on selection of mechanistically and structurally similar analogues. Along with the selection of analogues, the Toolbox provides a functionality (called Alert performance), which could be used to estimate the percentage of positive and negative analogues available within the Toolbox databases and sharing the same mechanism(s) of interaction with macromolecules as the target chemical. The third pillar of the QSAR Triad is associated with the expert approval of the results obtained in the previous stages, which is an important step for verification of final prediction based on external knowledge. In general, the suggested reliability workflow is applicable for any (eco)toxicological endpoint with known mode of action and clear mechanisms of interaction with macromolecules.

4.01.P-Mo281 Exposure Driven Environmental Risk Assessment - A Case Study on Fragrance Materials.

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Fragrance materials are ubiquitous in the environment due to pervasive uses in cosmetics, consumer products, and other daily use items. Over the past 20 years, the Research Institute for Fragrance Materials (RIFM) has been the leader in conducting environmental risk assessments on more than 3,000 fragrance materials. It has been with this mission that RIFM has developed an updated three-tiered framework that utilizes exposure-based modeling for assessing and ensuring the safe use of these materials. The recent updates to this framework allow for the more accurate assessment of fragrance materials by employing new approach methodologies (e.g., mode-of-action based ecological thresholds) to limit the use of animal testing, covering more geographical regions, and yielding realistic exposure concentrations (e.g., using updated IFRA Volume of Use surveys). The RIFM Environmental Risk Assessment determines exposure concentrations by using now available data on region-specific water usage, wastewater treatment models, river dilution, and measured biodegradation and ecotoxicity data. In addition, a hazard assessment is evaluated alongside the risk assessment, to ensure the safety of the fragrance material. The hazard assessment is conducted by using available measured or modeled persistence, bioaccumulation, and toxicity data. Following the completion of a risk and hazard assessment of a fragrance material, a four-to-five-year maintenance cycle begins which allows RIFM to re-evaluate the material to maintain and update RIFM's previous conclusion. This poster highlights a case study in which the step-by-step approach of exposure-based risk assessment of a fragrance material is employed. By using RIFM's conclusions drawn from the updated framework, manufacturers and regulators are able to create accurate science-based decisions to ensure the safe use of fragrance materials.

4.01.P-Mo282 New Training Course on Environmental Safety for Cosmetic Safety Assessors

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Cosmetic safety assessors are increasingly confronted with questions relating to the environmental safety of cosmetic products.

Trained according to traditional curriculum in chemistry, human toxicology, dermatology and cosmetic legislation, they often lack the necessary background to address these emerging concerns.

To address this gap, a new online training course module will be launched in 2026 to include environmental safety assessment into the qualification scheme for cosmetic safety assessors.

Developed in collaboration with industry professionals and association experts, the course provides fundamental knowledge on environmental safety assessment of chemicals used in cosmetic products. It introduces essential concepts, regulatory frameworks, and practical methodologies relevant to evaluating the environmental impact of cosmetic ingredients.

4.02 Beneath the Surface: Advancing Soil Ecotoxicology and Risk Assessment for Realistic and Sustainable Ecosystem Protection

4.02.T-01 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:

- to estimate the contribution of migration from untreated areas to the recovery of strongly damaged earthworm populations and

- 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.

4.02.T-02 From In vitro to Field: Comparison of Results from Tests of different Levels of Realism for Arbuscular Mycorrhizal Fungi

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Arbuscular mycorrhizal fungi (AMF) form mutualistic associations with many crop species and can play an important role in nutrient uptake and soil health. AMF were identified as focal organism group for future soil risk assessment [EFSA, 2017]. Our objective is to compare own and literature results of lab experiments, greenhouse-based, semi-field and field tests and to evaluate effects of model substances on AMF colonization under different exposure conditions. A potential tiered testing and risk assessment approach is discussed.

From three separate research groups, pre-symbiotic laboratory experiments with spore germination tests in medium acc. to ISO 10832:2009, performed with several fungicides and on different AMF species were analysed. Only the two AMF species *Rhizophagus intraradices* and *R. irregularis* showed a sufficient dose-response and can be considered reliable for inclusion into regulatory testing. Overall the ISO 10832 medium test with the right species is well suitable to differentiate under worst case conditions regarding effects on spore germination and hyphae elongation providing a good tier 1.

Performing tests that include the symbiosis between AMF and a host plant represents a major step towards more realistic conditions. In symbiotic tests performed in small pots in the greenhouse with barley and maize plants, a differentiation between the substances dependent on dose, route and timing of exposure was evident. The magnitude of effects was reduced compared to the medium tests. These test systems can be considered an intermediate-tier (tier 2) test systems between lab and field but still using a defined AMF species.

In a semi-field test with natural AMF population and natural soil in larger containers, with the exception of one product, the tested fungicides showed no significant effect on the mycorrhization process within the host plant. This semi-field test could act as possible tier 3 or higher-tier test system, still below a full field test design.

Ecotoxicological data from field-tests with AMFs, providing the highest tier, are limited. Available literature indicates that effects observed in the lab and greenhouse are attenuated in the field.

In conclusion, our results show that test designs with different levels of realism are available, providing sufficient sensitivity and clear dose-dependent effects for AMF. These could be used to develop a tiered testing scheme for AMF.

4.02.T-03 Experimental Validation of Mixture Toxicity Models for Pesticides in Soil

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Pesticides are widely applied to protect crops, yet their biologically active nature can harm non-target soil organisms. Current risk assessments focus on single substances, ignoring realistic mixtures that persist in agricultural soils for decades. This gap raises concerns about cumulative effects on soil fauna, such as springtails, which play a key role in nutrient cycling. Our study aimed to validate mixture toxicity models for terrestrial ecosystems experimentally, specifically the concentration addition (CA) model, which is established for aquatic systems but unvalidated in soils. We selected five commonly detected pesticides, two herbicides, two fungicides, and one insecticide, based on Swiss soil monitoring data and toxicity profiles. Using OECD232 reproduction tests with *Folsomia candida*, we assessed single formulations and mixtures of five formulations at environmentally relevant concentrations. All soil treatments (8 treatments for single substances and 21 for mixtures) were measured at the start and at the end of the test. This study is the first study to track a complex mixture of pesticides in springtails. Internal pesticide concentrations, for all 5 active substances and two metabolites, were measured to evaluate uptake and elimination over 28 days and analyzed with a Toxicokinetic (TK) model in the Build Your Own Model (BYOM) platform. Results indicate that CA predictions align closely with observed mixture effects, suggesting its applicability for soil organisms. These findings support the development of mixture-based risk assessments, improving ecological protection under real-world exposure scenarios.

4.02.T-04 Entry of Pesticides From the Air Into Soils far Away From Agricultural Areas as Potential Stress Factors for Soil Organisms

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The atmospheric transport of pesticides is an area of growing concern due to environmental entries at larger distances from agricultural land. Pesticide deposition (wet and dry) was investigated monthly over a period of one year at eight locations in agricultural and non-agricultural areas in North Rhine-Westphalia, Germany. A total of 18 active substances and metabolites were detected in self-constructed bulk samplers. At all sites, there were inputs not only in spring/summer, but also in autumn/winter. At non-agricultural sites, fewer substances and lower deposition rates were found. The herbicides prosulfocarb, flufenacet, terbuthylazine and its metabolite terbuthylazine-desethyl, as well as prothioconazole-desethyl were detected at all locations. In order to determine

the residues of pesticides deposited on the soil, samples were taken from the upper soil layer at one selected agricultural and one non-agricultural site. Sampling took place in June, September, November and February of the following year. All ten pesticides analysed were detected in the upper soil layers (0-2 cm and 2-10 cm). The highest concentrations were measured in June, but residues were detected at all sampling times, confirming persistence of pesticide residues in the soil. The results were used to calculate the mixture toxicological stress for soil organisms (earthworms, springtails and predatory mites), with earthworms exhibiting the highest stress. The drivers of the mixture toxic stress were terbutylazine, terbutylazine-desethyl, fluopyram and prothioconazole-desethio. In summary, atmospheric deposition is a significant entry route for pesticides into soils and subsequently a potential stressor for soil organisms. As protective approach for determining mixture toxic stress levels, soil concentrations can be estimated from depositions rates and used for calculating stress potential, which was also done with these data. However, field data show a more realistic picture as it includes residues of persistent substances and as it covers all further pathways of environmental fate of these substances. The results indicate the times of the year, which are important for measuring pesticides introduced via deposition. This will improve planning of future monitoring campaigns. In addition, the results indicate those pesticides, which are drivers of mixture toxicological stress for soil organisms.

4.02.T-05 Copper in Plant Protection: Long-Term Field Evidence on Earthworm Recovery and Soil Accumulation

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Copper-based plant protection products (PPPs) are essential in organic and integrated farming due to their broad-spectrum efficacy and lack of resistance development. However, copper does not degrade and can accumulate in soils, posing risks to soil organisms. EU renewal in 2018 highlighted high earthworm risk at Tier 1 based on chronic lab studies. A unique long-term (ongoing since 2003) field study in two sites in Germany, on grassland assessed three rates (4, 8, 40 kg/ha/year) and derived a NOAEC at 4 kg/ha/year considering only transient effects on earthworm abundance and biomass. Results support restricted use conditions: a maximum of 28 kg/ha over 7 years (24 kg/ha/year) with mitigation measures, ensuring no unacceptable environmental effects.

In autumn 2003 a GLP compliant earthworm field study was started on two grassland field sites located in Southern Germany. Naturally occurring field populations of earthworms present at the field sites were evaluated. The test item Copper hydroxide WP (500 g Cu/kg) was applied at nominal target rates of 4 (T1), 8 (T2) and 40 (T3) kg a.s./ha/year divided into three applications per year (e.g. for T1 an application in October, December and March at 1.5, 1.5 and 1 kg a.s./ha, respectively). T3 was not applied further than the 7th year. Following the demonstration of population sensitiveness and toxic reference efficiency, this was not applied after 8 years. Earthworms were sampled regularly to assess any effects on the earthworm population and to determine copper residue levels. Soil samples were taken and analysed for total and bioavailable copper residue levels in two different soil depths (0-5 cm and 5-30 cm). Statistical analysis were performed on the obtained results to demonstrate significant differences between test item treatments and control along the years.

Across several years of monitoring, earthworm populations at two field sites remained generally high, with only transient treatment-specific reductions. 4 and 8 kg/ha/year showed minimal long-term impact, while 40 kg/ha/year caused marked but partially reversible effects on certain taxa. Soil copper concentrations declined slowly in surface layers but remained elevated compared to baseline. Earthworm residues decreased over time, indicating limited bioaccumulation. Overall, the study shows gradual recovery of earthworm communities and stabilization of copper residues, with persistent localized effects at high application rates.

4.02.P Beneath the Surface: Advancing Soil Ecotoxicology and Risk Assessment for Realistic and Sustainable Ecosystem Protection

4.02.P-Th125 Modelling the impact of earthworm movement on pesticide exposure estimates for row crops

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Current Environmental Risk Assessment (ERA) of pesticides relies mainly on plot-scale approaches and often overlooks both landscape processes and organism behaviour, limiting realistic exposure estimates for soil organisms. This shortcoming is particularly critical in intensively treated systems such as vineyards, where spatial heterogeneity and species traits influence contact with contaminants. This study investigates how earthworm movement alone can shape exposure patterns under identical contamination conditions. We developed an individual-based model to simulate the movements of two ecologically contrasted earthworm species: the endogeic *Aporrectodea caliginosa* (low mobility, soil-confined) and the anecic *Lumbricus terrestris* (higher vertical and horizontal mobility). A spatially heterogeneous contamination pattern was implemented to represent localized soil hotspots found in row-crop systems, and this same contamination layer was applied to both species. As a first proof-of-concept, simulations were run under a fixed “worst-case” configuration with persistent hotspots, while additional scenarios accounting for temporal dissipation and degradation processes or ignoring spatial heterogeneity are in development. For each individual, we calculated a Potential External Exposure Index, defined as the cumulative sum of pesticide concentrations in all grid cells visited along the individual’s trajectory. This movement-based index is a proxy of cumulative external exposure, allowing comparison of species-specific movement. Under this configuration, pronounced interspecific differences were observed. *Lumbricus terrestris* displayed higher and more variable Potential External Exposure Index values than *A. caliginosa*. Its greater mobility and frequent horizontal and surface movements increased the likelihood of encountering and revisiting highly contaminated zones, raising exposure and inter-individual variability. In contrast, the more restricted and soil-confined movements of *A. caliginosa* resulted in lower and more homogeneous exposure patterns. These findings demonstrate that exposure is not solely determined by environmental concentration levels, but is also strongly modulated by ecological traits such as movement behaviour and habitat use. Ultimately, this framework supports the future coupling of the earthworm models with the MIPP pesticide fate model to generate spatially explicit and ecologically realistic exposure estimates for ERA in row-crop systems.

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4.02.P-Th126 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 7 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 2026.

4.02.P-Th127 Applying the 2023 Central Zone Guidance: Challenges in Soil Analytical Verification for Rapidly Degrading Actives

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In the European Union, the assessment of soil risk for plant protection products has historically relied on the Terrestrial Guidance Document (SANCO/10329/2002). This framework calculates toxicity exposure ratios

(TER) by comparing laboratory-derived toxicity endpoints, typically based on nominal test concentrations, with modelled maximum predicted environmental concentrations in soil (PEC_{max}). While this approach has provided a standardized method for evaluating potential risks to soil organisms, it does not account for the complexities introduced by substances that degrade rapidly in soil.

The 2023 Central Zone Working Document introduces a novel approach specifically targeting active substances with DT90 values shorter than the standard test durations for key soil invertebrates—earthworms, springtails, and soil mites (56, 28, and 14 days, respectively). Under this guidance, analytical verification of soil concentrations throughout the study is required, and when measured concentrations fall below 80% of nominal, endpoints must be expressed as time-weighted averages (TWA) or geometric means. This methodological shift has significant implications: TER calculations now combine modelled PEC_{max} peaks with long-term measured endpoints, potentially leading to overly conservative risk predictions that may not reflect real-world exposure scenarios. In the absence of available intermediate-tier refinements, this approach may increase the need for extensive, complex field studies to satisfy regulatory requirements.

This poster presents a case study comparing soil ecotoxicological datasets evaluated under the traditional SANCO (2002) approach—where endpoints were derived from nominal concentrations—with those assessed under the 2023 Central Zone framework, incorporating analytically verified concentrations. The analysis explores how different exposure metrics (PEC_{max} versus PECTWA) and sampling strategies influence TER outcomes and highlights the practical challenges of implementing the new guidance. The poster also discusses practical testing challenges and potential solutions for a more pragmatic, scientifically robust soil risk assessment framework that balances regulatory rigor with feasibility.

4.02.P-Th128 Revising guidance on pesticide risk assessment for soil organisms – considerations from an user perspective

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Under Regulation (EC) No 1107/2009, pesticide approval requires demonstrating the absence of unacceptable environmental effects. For soil organisms, the EFSA has to review the soil risk assessment currently based on the terrestrial ecotoxicology guidance (SANCO/10329/2002). CropLife Europe (CLE) provides considerations to support EFSA and national authorities in developing an aim-oriented, pragmatic and protective soil risk assessment. This poster outlines the scientific investment projects undertaken by the CLE ad-hoc soil expert group from the past years, and presents a set of user-oriented priorities or ‘wish list’ to be considered in the development of an updated soil risk assessment guidance.

The conclusions from the CLE soil group have led to the following recommendations:

? Alignment between the new EFSA (2017) soil exposure guidance and the future soil risk assessment is essential, without introducing an unjustified increase in conservativeness[1].

? The implementation of workable intermediate tier options should be promoted once the relevant data gaps have been addressed[2], and

? the inclusion of additional organism groups should only be considered if validated test protocols (including higher-tier options) are available, which is currently not the case, e.g., for mycorrhizal fungi[3].

? Assessment factors in a tiered risk assessment scheme should be based on data-based calibration following a transparent methodology[4].

? Fixed effect thresholds (e.g., 10%) for (semi-)field studies are unrealistic and scientifically unreasonable. Relevant threshold values can only be considered biologically relevant when they are above typical occurring natural variabilities[5] and species-specific responses. Instead, medium to large temporary effects should be acceptable for soil-function-relevant groups if recovery occurs within an agreed timeframe and effects are outside natural occurring variation[6].

The poster aims to serve as a basis for scientific discussion and foster the development of a data-based, practical, and cost-effective approach that strikes a balance between food security and the protection of soil fauna in agricultural landscapes.

[1]Schimera et al (2024), JESH B 59(4) pp 170-182; [2]Ernst et al (2023) IEAM 20(3) pp 780-793; [3]Sweeney et al (2022) ETAC 41(8) pp 1808-1823; [4]Christl et al (2016) IEAM 12(4) pp 643-650; [5]Schimera et al (2025) IEAM in press 10.1093/inteam/vjaf171; [6]Pamminger et al (2021) IEAM 18(5) pp 1423-1433.

4.02.P-Th129 Development of workable Specific Protection Goals for pesticide risk assessment for soil organisms

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Under Regulation (EC) No 1107/2009, pesticide approval requires demonstrating the absence of unacceptable environmental effects. For soil organisms, the European Commission has mandated EFSA (EC Mandate M-2024-0086) to review the soil risk assessment methodology as part of revising the terrestrial ecotoxicology guidance document (SANCO/10329/2002). CropLife Europe (CLE) provides considerations to support EFSA and national authorities in developing scientifically robust and operational SPGs for future risk assessment. SPGs should be defined for key ecosystem services (ESS) delivered by non-target soil organisms in production and non-production areas, distinguishing between primary and supportive ESS. For in-field areas, the primary ESS—agricultural production—must remain central, as acknowledged in EFSA (2017)¹, irrespective of potential conflicts with other ESS (e.g. biodiversity). Therefore, trade-offs between ESS should be explicitly addressed, prioritizing primary ESS for decision-making to ensure food security under increasing global pressures. SPG setting is only feasible for organism groups with established test protocols (earthworms, collembola, mites). While for newly proposed taxa, data generation is a prerequisite. Fixed effect thresholds (e.g., 10%) should be omitted as they are scientifically unjustified and impractical for (semi)field studies due to high natural variability². Overmore, Pamminger et al (2021)³ indicate that even large transient effects on abundance may not impair critical soil functions such as organic matter degradation. Therefore, medium to large temporary effects should be acceptable if recovery occurs within an agreed timeframe and within typical occurring natural variability, requiring clear definitions and methodologies. For off-field areas, diverse land uses and overlapping regulations (e.g., Sustainable Use Directive, Habitats Directive, Nature Restoration Regulation) complicate SPG development. Establishing realistic SPGs at landscape scale requires definition of land use scenarios and an understanding of spatial and temporal variability of soil communities within these—a current research gap. CLE emphasizes that SPG development must integrate ecological science, sustainability objectives, and practical feasibility to maintain agricultural productivity while meeting regulatory protection goals.

¹ EFSA Journal 2017;15(2):4690; ² Schimera et al (2024) IEAM in press 10.1093/inteam/vjaf171; ³ IEAM 18(5) pp 1423-1433

4.02.P-Th130 Ecological Profiling of Taxa for Risk Assessments

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Ecological profiling of taxa can improve pesticide risk assessment by integrating species-specific ecological traits—such as habitat use, feeding behaviour, and life-cycle characteristics—into exposure and effect evaluations.

Springtails (Collembola), among the most abundant soil arthropods and indicators of soil health, show strong spatial and temporal variation linked to humidity, organic substrates, and microclimatic conditions. These dynamics influence the representativeness of field samples, which is essential for assessing the effects of plant protection products (PPP) on springtail communities.

In a pilot study, we examined (relatively) small-scale spatial dynamics in springtail composition, and how these shape the observed effects of a PPP treatment on the populations. Using a checkerboard design, we assessed how patchy PPP applications translate into direct and indirect impacts on springtail populations. Because springtails move horizontally as well as vertically, we used aspirator (soil-surface) and mine trap (in-soil) sampling methods, to capture the community dynamics in three dimensions, with a special focus on identifying potential source-sink dynamics of springtails and their spatial ranges.

Besides spatial dynamics, springtail communities show temporal dynamics, e.g. seasonal or even diurnal. We investigated, if we can find remarkable differences in springtail community composition between climatic regions (Central vs. Southern). We focused on identity, abundance and ecological types of taxa: euedaphic, hemiedaphic, epedaphic. Particular focus was given to short-term (diurnal) temporal variation in community composition especially in warm dry habitats. These can influence the representativeness of field samples, which are critical for assessing the effects of PPPs. For example, using aspirator sampling during the day in a warm dry environment, might not lead to an exhaustive sample of the springtail community present in that habitat, since springtails move between soil layers (vertical stratification).

Through this comparative approach, we aim to develop tailored sampling strategies for Collembola that account

for spatial and temporal ecological dynamics and thereby enhance the reliability and ecological relevance of PPP risk assessments under different climatic conditions.

4.02.P-Th131 Implementing Arbuscular Mycorrhiza Fungi in an Environmental Risk Assessment – Case Study with Benomyl considering Effects on Spore Germination and Symbiosis

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Arbuscular mycorrhizal fungi (AMF) are ubiquitous in terrestrial ecosystems and form symbioses with most plants, thereby can enhance nutrient uptake, pest tolerance, and yield. Despite their ecological importance, AMF have been recognized as non-target soil organisms relevant for environment risk assessment (ERA) of plant protection products (PPP) within the EFSA soil opinion (2017). The current OECD nitrogen transformation test (OECD Test Guideline (TG) 216), used to assess PPP effects on soil microorganisms, does not address impacts on AMF.

To close this gap, the UBA project MICROSOIL proposes to include a spore germination test (ISO 10832) as a Tier 1 screening method. However, this method does not capture the symbiosis between AMF and host plants. Therefore, a symbiosis-test-method was developed within the UBA project ERAMYC for assessing the effects of PPP on the symbiotic phase of AMFs. In our study, we observed the effect of a PPP in artificial and natural soil on AMF using both methods.

Preliminary experiments using *Funneliformis mosseae* confirmed sufficient spore germination in artificial soil and the reference soil RefeSol 02A, which were selected as substrates for all further test systems. The fungicide Benomyl was used as a reference substance and showed a clear dose–response relationship on spore germination in two independent experiments. In the spore germination tests an EC₅₀ of 4.5 mg/kg dry weight (dw) soil in artificial substrate and of 3.23 mg/kg dw soil in natural soil were determined. The ISO 10832 states that the EC₅₀ should be between 1 and 10 mg/kg dw soil and therefore the results show that the test worked well in the natural soil.

In the subsequent Tier 2 symbiosis test, *Allium ampeloprasum* (leek) serves as the host plant with *F. mosseae* as the fungal partner. After a 56-day exposure, endpoints include intraradical colonization, extraradical mycelium production, and plant biomass. In parallel, the OECD TG 216 test is conducted to compare AMF sensitivity to the standard soil microbial assay used in ERA. The experiments on the symbiosis of AMF were initiated in September/October 2025. The tests will be terminated and evaluated in January 2026, the results will be presented on the poster.

Results from all tests will be analyzed to evaluate sensitivity, ecological relevance, and potential regulatory applicability.

4.02.P-Th132 AM fungi – which species should be tested in laboratory tests for soil ecotoxicological risk assessment of plant protection products and their active substances

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Glomeromycota comprises the arbuscular mycorrhizal (AM) fungi, which occur in all agricultural soils worldwide with varying abundance and richness depending largely on climate, soil, crop/vegetation and land use intensity. They are of major significance for ecosystems, as they improve plant growth, suppress pathogens and enhance soil fertility & stability and water infiltration. AM fungi comprise > 350 species divided into 3 classes, 9 orders, 26 families and >60 genera. They are at risk due to excessive soil cultivation or application of plant protection products. We ask, which taxa are appropriate to be tested in dose-response ecotoxicological laboratory experiments with plant protection products (PPP) and their active substances (AS). Different AM fungal taxa are clearly seasonal or not, have specific life cycles, hyphal networks and spore formation characteristics. Spores, or ‘fruit-bodies’, are differentiated within weeks, only after months or years, and sometimes not at all. They are formed singly, in clusters or huge agglomerations, in soils or preferably in plant roots, either perfectly mature, or as early aborted spores without any germination capacity and function. Estimations can be made about the beneficial performance of well-studied AM fungal taxa or about their sensitivity in different soil management or cropping systems and agricultural land use intensities.

Glomeromycetes species have increased beneficial potentials, with respect to plant growth promotion, plant health, soil structure formation or soil health. Some species are commercially available, for bio-remediation, as bio-pesticides or bio-fertilizers. Genera with most positive ecological value, or highest benefits for plants or soils either belong to Glomerales, Entrophosporales, Diversisporales, Acaulosporales or Gigasporales. In

contrast, Archaeosporales or Paraglomerales spp. were rarely shown to have positive effects on plants or soils, while Pacisporales and Sacculosporales spp. have rarely been cultured. It is however a black box, which of all these taxa might be more or less sensitive to herbicides, insecticides or even fungicides. Here, we aim to identify those AM fungi with different traits, properties and ecological benefits, but also most suited for ecotoxicological tests with AS or PPP. Our criteria are based on the knowledge on fungal structures, culturability, significance as non-target organisms and especially as ‘improvers’ of plant growth & health and soil fertility.

4.02.P-Th133 Toxicity of Azoxystrobin to Arbuscular Mycorrhizal Fungi Assessed across Soil, Drench, and Foliar Exposures in a Controlled Intermediate-Tier Greenhouse Test System

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Arbuscular mycorrhizal fungi (AMF) are widespread plant symbionts that play key roles in nutrient acquisition, soil functioning, and crop performance. Their ecological relevance has been recognised by the European Food Safety Authority (2017), which designated AMF as a focal non-target organism group for future soil risk assessment. Current testing is largely based on ISO 10832:2009, a Tier 1 in vitro assay targeting spore germination. A second Tier would need to include AMF responses during functional symbiosis. This study aimed to establish a greenhouse-based test system to evaluate the effects of a model substance on AMF colonization under defined exposure routes, providing an intermediate tier between laboratory and field studies.

A standardized pellet-based *Rhizoglyphus irregularis* inoculum ensured precise and homogeneous AMF input into natural agricultural soil planted with maize. Plants were grown under controlled greenhouse conditions. The commercial azoxystrobin formulation AMISTAR® (250 g/L) was applied (i) via soil mixing at planting, (ii) soil drenching, or (iii) foliar spraying with soil protection at single, double, and 20-fold field rates. A full-factorial greenhouse experiment with five biological replicates was conducted in a randomized design. Plants were sampled 15, 30, and 45 days after planting. AMF colonization was quantified by colonization frequency (F%), indicating the proportion of colonized root segments, and colonization intensity (M%), describing colonization within affected segments. The abundances of arbuscules, vesicles, and intraradical hyphae were assessed to characterise structural composition.

At day 15 there was no AMF colonization, independent from azoxystrobin. At days 30 and 45, marked dose-dependent reductions in both F% and M% were observed, with the highest dose eliminating colonization. Drench applications allowed partial colonization but reduced F% and M% compared with the control, whereas foliar exposure resulted in only mild suppression. Structural analysis showed differences in sensitivity among AMF structures: arbuscules and intraradical hyphae declined most strongly, while vesicles were less affected and more variable.

This greenhouse-based approach offers a practical intermediate-tier test system that increases ecological realism and allows early observation of colonization and recovery, while keeping variability low enough to derive statistically robust endpoints.

4.02.P-Th134 Toxic Cocktails in Soils : Assessing Fungicide-Insecticide Mixture Effects on Earthworm Reproduction

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Soils are crucial reservoirs of biodiversity and providers of essential ecosystem services, yet they are increasingly threatened by agricultural intensification and pesticide use. Pesticide residues frequently persist in soils as complex mixtures, whereas environmental risk assessments typically focus on single compounds, thereby overlooking potential mixture effects. Earthworms play a key role in soil functioning and are particularly vulnerable to pesticide contamination. We investigated the combined effects of two persistent compounds frequently detected in agricultural soils, epoxiconazole (a fungicide) and imidacloprid (an insecticide), on life-history traits of *Aporrectodea caliginosa*. Based on their known modes of action, synergistic

interaction could theoretically occur, with triazole-mediated inhibition of detoxification pathways potentially enhancing imidacloprid toxicity. In a first experiment, each compound's relative potency was estimated by characterizing the dose–response relationship for cocoon production, following an adaptation of OECD guideline 222. In a second experiment, mixture effects were assessed using a fixed-ratio ray design across five concentration ratios and 36 conditions ($n = 8$ per condition). Adult growth and cocoon production were monitored for 28 days, followed by hatching assessment over 56 days. Both pesticides exhibited significant toxicity. Imidacloprid was markedly more potent ($EC_{50} = 0.55$ mg/kg) than epoxiconazole ($EC_{50} = 126.8$ mg/kg). Mixture analyses using Jonker's interaction models showed significant deviation from concentration addition suggesting synergistic interactions. This conclusion is in accordance with potential metabolic interactions reported in the literature. Environmental risk assessment revealed concerning exposure scenarios, particularly for imidacloprid, as maximum detected soil concentrations (0.16 mg/kg) approach effect thresholds with safety margins of only 1.8 to 3.4 fold, below typical regulatory factors. A key knowledge gap remains as to whether the hypothesized metabolic interference mechanisms occur in earthworms. Dedicated toxicokinetic and biomarker investigations are therefore required to quantify internal exposure and assess the plausibility of such mechanisms.

4.02.P-Th135 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 a key component of the test battery for environmental risk assessment of plant protection products in the soil compartment.

Although the validity criterion requires a minimum of 100 juveniles per control replicate after the 28-day test period, actual juvenile numbers are often much higher—frequently exceeding 1000. After extraction from the test soil using dyed black water, the white adult and juvenile springtails float on the water surface and can be counted either directly under a binocular microscope or via photographs taken from the water surface. Both approaches, however, are time-consuming, labor-intensive, and prone to human error.

To streamline, accelerate, and standardize this process, the software FOLSOMIACOUNTER was developed to enable automated juvenile counting from photographs. An integrated AI-based feature also estimates body size for both adults and juveniles, supporting the detection of sublethal effects beyond reproduction. Over recent years, the AI has been continuously trained and refined using photographs of varying quality.

We present a comparison of manual versus software-based juvenile counts using photographs from annual toxic reference item studies (2023–2024), which were excluded from the software's training dataset. The poster discusses results in terms of:

- (a) time savings achieved,
- (b) counting accuracy,
- (c) impact on the main ecotoxicological endpoint (EC_{50}), and
- (d) potential of body size measurement as an additional sublethal parameter.

In addition, the current extension of this approach to earthworm counting, and the associated challenges will also be presented.

4.02.P-Th136 Neonicotinoid insecticide residues in agricultural soils in Germany

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Neonicotinoids are among the most widely used insecticides worldwide. The use of some neonicotinoids was largely banned in the European Union several years ago due to adverse effects on the nervous system of bees and other pollinators and, in the case of thiacloprid, due to carcinogenic and reproductive toxic effects on humans. However, due to the very long half-lives of some of these active substances in soil, significant and long-lasting contamination of agricultural soils on which they have been applied is expected, despite restrictions on their use.

Between 2021 and 2024, a Germany-wide study was conducted in which soil samples were taken from 100 areas under agricultural use at soil depths of 0–5 cm and 5–20 cm. The aim of the study was to record the level of chemical residues in agricultural soils. The subproject presented here investigated the soil residues of

neonicotinoids. For this, a highly sensitive analytical method was developed using QuEChERS processing and UHPLC-MS/MS detection with limits of quantification (LOQ) of 10 ng/kg for the five most important active substances within this group of substances: imidacloprid (IMI), thiamethoxam (THIAM), clothianidin (CLO), acetamiprid (ACE), and thiacloprid (THIAC). The results show extensive contamination of the soils examined, with detection rates > limit of detection (LOD) of 17% (ACE), 44% (THIAC), 36% (THIAM), 95% (IMI), and 86% (CLO) in arable land, for example. The spatial distribution of residues was examined with respect to the main land-use types (arable land, wine-growing areas, orchards, and grassland), geographical distribution, soil depths, and temporal trends. Despite comprehensive bans on the use of four of the investigated active substances, the data demonstrate the persistence and widespread distribution of neonicotinoid residues in agricultural soils across Germany. The results serve as a basis for assessing the actual environmental risk in the soil from the cumulative chemical contamination. In addition, the findings help to close existing gaps in the risk assessment of chemicals and form the basis for a prospective risk assessment with regard to adverse effects on soil biodiversity.

Disclaimer/Disclosure:

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4.02.P-Th137 Influence of Hydrogel Soil Amendments on Lambda-Cyhalothrin Toxicity in Two Standard Soil Mesofauna Bioindicators

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Hydrogels are increasingly incorporated into agricultural soils to improve water retention and crop resilience under drought, yet their interactions with pesticide fate and soil invertebrate toxicity remain poorly understood. This study investigates how a representative hydrogel influences the toxicity of the pyrethroid lambda-cyhalothrin to two standard soil mesofaunal bioindicators, *Folsomia candida* and *Oppia nitens*. We hypothesize that hydrogel supplementation will reduce effective exposure by enhancing sorption and altering soil water dynamics, thereby mitigating toxicity relative to non-amended soils; moreover, effects may differ between the natural and artificial soils due to contrasting textures and organic matter contents. To do this, the experiment was conducted using one hydrogel type, six pesticide concentrations (including control), and two contrasting soils: a natural loamy sand and OECD artificial soil. Hydrogels were homogenized into each soil at an agronomically relevant rate and equilibrated 7 days prior to pesticide application. Standardized 28-day reproduction tests were performed separately for *F. candida* and *O. nitens* following OECD 232 and ISO 23266 respectively, to assess adult survival, reproduction, and growth. The soil moisture, pH, and lambda-cyhalothrin residues in soil and porewater, will be measured, to quantify hydrogel-driven changes in pesticide bioavailability. Data will be analyzed using GLMs, two-way ANOVA, and correlations linking biological responses with measured residues. Dose-response of lambda-cyhalothrin will be assessed using non-linear regressions in R. This study will help to see the combined effects of soil amendments and pesticide stressors on soil mesofauna, informing sustainable pesticide management and soil-amendment strategies under climate-resilient agriculture. The test is ongoing.

4.02.P-Th139 Eco-Toxicological Assessment of Hydrocarbon-Contaminated Soil Recovery Using Plant and Earthworm Bioassays

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Hydrocarbon contamination poses significant risks to soil health, yet chemical analysis alone cannot reliably assess ecological recovery because it overlooks bioavailability and biological responses. This study aimed to identify sensitive and reliable bioassays for evaluating the recovery of kerosene- and diesel-contaminated soils using plant and earthworm responses. Three soil types were amended with 1% hydrocarbons and subjected to non-incubated and incubated treatments to simulate freshly contaminated and remediated conditions. Bioassays included seed germination, root elongation, shoot height, germination index for mustard and wheat, and 14-day survival of earthworms. Results showed strong soil-dependent response patterns. Mustard root elongation was highly sensitive to contamination and effectively detected residual toxicity, while wheat shoot height was the best indicator of soil recovery. In contrast, seed germination showed low sensitivity across treatments. Earthworms exhibited complete mortality in freshly contaminated soils but could not distinguish between incubated and non-incubated treatments, indicating persistent toxicity. Overall, plant growth parameters were the most informative indicators of both contamination levels and recovery status. The study demonstrates that a multi-bioassay approach offers a more comprehensive and ecologically relevant assessment of hydrocarbon-

contaminated soil recovery compared with chemical analysis alone, supporting improved ecological risk assessment and remediation evaluation.

4.02.P-Th140 Is biochar toxic to soil organisms? Standard tests with enchytraeids, springtails and earthworms

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The few existing studies of biochar impact on soil fauna report varying effects, i.e. both negative and positive effects have been seen. As biochar characteristics vary not only between feedstocks, but also between batches, the variation in organism responses may not come as a surprise. Nevertheless, assessing possible risks to soil organisms is crucial to secure safe use of biochar, which is, by some politicians and administrators, seen as an obvious tool for combining climate change mitigation and improvement of agricultural soils.

As an initial assessment of the possible toxic effects of biochar made from straw and biogas digestate, respectively, OECD reproduction tests were performed with the enchytraeid *Enchytraeus albidus*, the spring-tail *Folsomia candida* and the earthworm *Eisenia fetida*, all organisms used in standard testing of e.g. pesticides. To ensure maximum exposure, biochar pellets were ground and mixed into the test soil. Based on results from a range-finding test, test concentrations in the range 0-10 and 0-100 mg/kg soil were applied. High biochar concentrations affect soil moisture, and to omit this factor from the test and concentrate on possible toxic effects, soil moisture levels were carefully adjusted for each biochar concentration level.

Adult survival of the three test species was not significantly affected by the tested biochar concentrations. The reproductive output of enchytraeids was slightly increased at the higher biochar concentrations, whereas no effects were seen on springtail reproduction. For the earthworms, high biochar concentrations seemed to delay the reproduction process; the total reproductive output (no. cocoons + no. juveniles) was hardly affected, but the ratio cocoons:juveniles was increased.

The outcome of this study suggests that further studies involving repeated biochar application, other feed-stock types and more agriculturally relevant conditions should be performed. Fortunately, a long-term field study has been established to do exactly that: At four experimental stations, four types of biochar will be applied for at least five years to study long-term effects on crop yield, soil physio-chemistry and leaching, and soil organisms. Apart from elucidating the potential toxicity of selected biochar products to soil fauna etc., the projects can also serve as basis for establishing procedures for future risk assessment of biochar.

4.02.P-Th141 Ecotoxicological Assessment of Excavation Material Contaminated by Munitions and Explosives Residues

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In Switzerland, as in other parts of the world, military activities such as training exercises, storage, and accidents have led to the contamination of soil with munitions and explosives, resulting in environmental pollution.

However, in this research project, primary ecotoxicological tests revealed very low toxicity in plant germination and invertebrate reproduction despite very high concentrations of metals, polycyclic aromatic hydrocarbons and explosives, exceeding Swiss remediation thresholds. The main theoretical explanation for this lack of effects is assumed to be the low overall bioavailability of these chemicals.

The ongoing aim of this research project is to bridge the gap between chemical analysis and ecotoxicological testing by focusing on bioavailability as the key driver of exposure. By adapting existing standardized soil ecotoxicity methods to enable the recovery of organisms after exposure, internal contaminant concentrations from exposed organisms and plants will be quantified and linked to their biological responses. In parallel chemical analysis of the total contaminants' concentrations in soil will allow to evaluate the link between total and internal concentrations, and how well these different fractions of contaminants correlate with ecotoxicological effects.

This integrated chemical-biological approach will help gain insights into bioavailability and help explain why high contamination levels do not necessarily translate into measurable toxicity. Future work may extend these methods to contaminated field sites, validating laboratory approaches through internal concentrations in field-collected organisms. Ultimately, this research aims to improve the assessment of risks posed by munition and explosives by focusing on the bioavailable fraction that truly drives ecological impacts.

4.02.P-Th142 BioDive4Soil – A systematic assessment of soil biodiversity

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The decline in biodiversity in conjunction with global change poses a severe threat to ecosystem functioning and long-term resilience. Soils host around 60 % of all species on earth but our knowledge of soil biodiversity across different land-use types and its drivers remains limited. Recognizing its importance, the German Environment Agency (UBA) has launched a high-impact research program in collaboration with the Fraunhofer-Gesellschaft and ten other institutions. The project is funded by the Natural Climate Protection Action Program (ANK) of the Federal Ministry for the Environment (BMUKN).

Here, we present an overview of the recently initiated German BioDive4Soil project – a large-scale systematic assessment of soil biodiversity. In BioDive4Soil, we will assess soil biodiversity across Germany, covering different habitats, land-use types, and soil regions. We will sample in near-natural ecosystems and across a gradient of increasing land-use intensity.

We will establish baseline knowledge of soil communities by determining morphological, functional, and molecular diversity of soil microorganisms and fauna. We will perform eDNA-metabarcoding and barcoding of individual species across different soil taxa to improve genomic reference data of soil organisms. We aim to identify soil biological reference values indicative for healthy soils and important drivers of soil community composition, such as land-use management, soil type, and pollution.

Taken together, BioDive4Soil aims to close existing knowledge gaps and to improve our understanding of the diversity of soil organisms in Germany, changes in their composition, their main drivers and possible approaches for the protection and restoration of soils and soil biodiversity.

To date, an initial draft of the overall concept for the baseline survey has been developed and is being continuously refined over the coming month in close collaboration with other national monitoring programs and with consideration of the European soil monitoring law. Areas were identified for an initial sampling campaign, and samples were taken from 10 out of a total of 250 sites in autumn 2025.

Disclaimer/Disclosure:

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4.03.P Chemicals and Biodiversity in Managed Landscapes: Systems Thinking, Spatial Scales and Sustainable Solutions

4.03.P-Th143 Rehabilitating contaminated soils via phytomanagement for circular bio-Based applications: A Belgian field Case

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As part of the IASIS project, the University of Liège is conducting a field case study in Wallonia (Belgium), a former industrial site contaminated with hydrocarbons and heavy metals. Funded by Horizon Europe and the CBE JU, IASIS is exploring phytomanagement as a sustainable strategy for the remediation of contaminated and saline soils across Europe.

The IASIS project advances phytomanagement strategies for rehabilitating contaminated and saline soils, with a particular focus on tailored microbial consortia. These approaches are tested and validated at both laboratory and field scales. Eight Field Case Studies (FCSs) have been established across Europe, four on contaminated sites and four on saline soils, where eight high-yielding, non-food industrial crops are cultivated using low-input, sustainable practices. The project supports the development of bio-based products through two main crop categories: oilseeds and lignocellulosic species, integrated into cascading biorefinery value chains.

The Belgian FCS involves cultivating three plant species on a 2-hectare site using low-input agricultural practices, including plant-based fertilizers, microbial consortia, and minimal mechanization. The field is

structured into crop- and treatment-specific plots, replicated across the site and over three growing seasons to ensure robustness and reproducibility of results.

First a pot experiment in greenhouse was conducted to select the soil amendments and test plant growth on contaminated soils collected from the field site. Following soil preparation, the first field trial was launched under low-input conditions. Soil health indicators, including microbial diversity and pollutant concentrations, have been evaluated. Biomass development had been monitored using drone imaging, and harvested samples are undergoing analysis to assess quality parameters relevant to downstream applications. A second growing season is scheduled for spring 2026. The poster will present data analyses from the pot experiment and the first growing season, along with preliminary observations from the second.

Disclaimer/Disclosure:

IASIS project GA101157430

4.03.P-Th144 Assessing Channelisation Impacts on Benthic Macroinvertebrate Assemblages of a Lowland Agricultural Catchment

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Following legacies of hydromorphological alteration, lowland rivers are highly threatened by multiple, often synergistic pressures including pollution, invasive species, and anthropogenic climate change. Hydromorphological degradation undermines river ecosystem integrity often through habitat homogenisation to the detriment of ecosystem services. The European Union Water Framework Directive was introduced partly to alleviate hydromorphological degradation and support surface water bodies in achieving 'good' ecological status.

The research project aimed to assess the impact of channelisation on benthic macroinvertebrate assemblages of a lowland agricultural catchment by comparing kick-sampled macroinvertebrates between good and poor morphology reaches. The study also included physicochemical site assessment using handheld sensors, Modular Physical River Habitat surveys, and field observations. There was an additional focus on invasive *Dikerogammarus* distributions within the catchment considering possible habitat homogenisation induced by channelisation for poor morphology reaches.

The results demonstrated macroinvertebrate assemblages were alike between morphology types, highlighting macroinvertebrates can recover from anthropogenic disturbances depending on local catchment characteristics. Well-established channelisation was identified as weakly influencing macroinvertebrate assemblages, especially where local restoration measures improved diversity of formerly degraded reaches with good colonisation potential.

It was concluded long-term monitoring, for which the scope improves with citizen science, is essential to fully understanding macroinvertebrate responses to hydromorphological change considering invasive species and anthropogenic climate change impacts.

This study valuably assessed multiple stressors acting on the study system. It examined how far habitat can be modified before ecosystem integrity is undermined and ecological effects are detrimental. It affirmed biological and physicochemical river health assessments remain highly relevant for river degradation evaluations, especially applied long-term, over multiple spatial scales to inform adaptive management, which can assist future management decisions to support ecosystem services. Research gaps addressed also included whether channelisation facilitated biotic invasions of *Dikerogammarus* within a lowland agricultural catchment and Site of Special Scientific Interest.

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4.03.P-Th145 Lithium mining could threaten hundreds of vulnerable and endangered species

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Electric vehicles (EVs) are being rapidly deployed as a technological solution to the climate crisis. Mineral extraction to supply EV battery production can cause serious ecological harm, threatening global biodiversity.

Lithium, a key EV battery component, is expected to experience an enormous mining boom. This increased mining could significantly threaten nearby species, potentially driving some to a higher extinction risk.

Using the IUCN Red List, we assessed how 400 operational and prospective lithium mines could threaten their surrounding species. First, we determined each mine's coordinates using company reports and academic literature. Then, we applied 50 km buffers around each site, representing the total area in which both direct and indirect mining impacts could harm local species. Next, we assessed how much the buffered mines overlap with each Red List species' range, from 0 to 100 %. We then weighted each overlap percentage by the species' extinction probabilities to compute threat scores for the mines. We also developed a global list of species that could be under the greatest threat from lithium mining, considering various scenarios for future mine construction. Additionally, using the Red List's categorisation criteria, we identified range-restricted species that could be at risk of being pushed to more severe threat categories than they are currently classified in.

We found that Australian lithium mines pose the greatest threat by a large margin. Globally, we identified up to 193 threatened species could be significantly impacted by lithium mines. Additionally, we noted at least 184 range-restricted species could face higher-than-expected extinction risks. Red List species ultimately represent one of many facets of global biodiversity. While mitigating harm to these species is critical, it is futile to attempt to protect them alone without also safeguarding the wider ecosystems they depend on. Future work on this topic should involve developing a broader, more robust framework to capture and mitigate the totality of each mine's potential impacts on surrounding ecosystems.

Disclaimer/Disclosure:

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4.03.P-Th146 What is biodiversity and why should we be measuring it?

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Businesses are facing increasing pressure from regulators and investors to disclose their impacts and dependencies on biodiversity. However, "biodiversity" is nebulously defined as all variability in the natural world, according to the United Nation's Convention on Biological Diversity. Not surprisingly, this has generated significant confusion over what businesses should be measuring – and how they should be measuring it. This presents a particular challenge for regulators seeking to understand the chemical thresholds necessary to protect the environment and its myriad processes. This poster will discuss the history of biodiversity as a concept, walk through recent attempts to synthesize and standardize biodiversity (e.g., TNFD, CSRD, GEOBON's Essential Biodiversity Variables), and examine how the new ISO Biodiversity Standard (ISO 17298) alters the playing field. In addition, we will revisit central research themes that are increasingly omitted from the conversation, including the relationship between biodiversity and ecosystem function, the need for biodiversity as insurance against future disturbances, and the overwhelming – albeit generally overlooked – importance of functional trait diversity.

4.03.P-Th147 The challenge of the ATAT indicator for the Global Biodiversity Framework: Good Intentions hampered by unscientific metrics

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The Kunming-Montreal Global Biodiversity Framework (GBF) is a recently adopted agreement that supports the Sustainable Development Goals and builds on the previous Strategic Plans of the Convention of Biological Diversity. There are several indicators that are relevant for agro-chemicals, among them those of the GBF Target 7. The overarching aim of Target 7 is to reduce pollution risks and their negative impacts on biodiversity and ecosystem functions to levels that are not harmful by 2030. This target is divided into reduction of nutrient pollution (Target 7.1) and reduction of risk from pesticides and hazardous chemicals (Target 7.2). The Aggregated Total Applied Toxicity (ATAT) is one of the proposed headline indicators (7.2) to measure progress

towards this specific goal focusing on pesticides. However, there has been no validation to determine the extent to which the methodology will result in an actual risk reduction, e.g. when one substance is substituted by another.

According to OECD and EFSA guidance, a methodology must be validated before used in a regulatory system. The first important step for validation of new approaches or model calculations is to conduct a verification, so the aim is reflected correctly in the mathematical equations. Already this first verification step fails for the ATAT since unsimilar units are added up to each other. Therefore this indicator is not scientifically sound nor the results easy to interpret. One of the further verification calculations indicates that the ATAT is sensitive to areas with low risk (high RTL) and not to the risk assessments areas which actually drive the risk to biodiversity. While there appears to be a disconnect between the ATAT indicator, the current toxicity assessments and actual effects on biodiversity, a more integrated approach could be a solution.

Disclaimer/Disclosure:

The authors work for companies selling agrochemicals.

4.04 Environmental Hazard and Risk Assessment of UVCBs and Complex Mixtures: From Scientific Challenges to Regulatory Solutions

4.04.T-01 How can simple data facilitate the assessment of data-poor complex substances? Application of baseline (Tier 0) data for UVCB evaluation

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The evaluation of substances of Unknown or Variable composition, Complex reaction products and Biological materials (UVCBs) is often complicated by the lack of a consistent, reliable and well-curated source of data. This can introduce challenges when attempting to conduct substance prioritizations, evaluations, or hazard/risk assessments. To address this gap, the UVCB Committee of the Health and Environmental Sciences Institute (HESI Global) developed a pilot database (the 'PULSE' database) of baseline ('Tier 0') data for 109 UVCBs. This contribution describes various use cases for data from PULSE, highlighting challenges associated with UVCB evaluation and the opportunities that Tier 0 data present to fill some of these data gaps. To demonstrate database functionality, two UVCB substances were selected, and data availabilities and limitations were explored across PULSE. Two case studies were then conducted to demonstrate the utility of Tier 0 UVCB data for data gap filling and substance evaluation, using PULSE as the primary source of data. In the first case study, Tier 0 data were used to group 14 metal naphthenates (based on structural characteristics and manufacturing process), and environmental fate data of 6 'data-rich substances' was read-across to fill data gaps for 8 data poor substances. This demonstrated that metal naphthenates may contain both (readily) degradable and non-degradable fractions and display low bioconcentration factors. In the second case study, available data for 88 UVCB substances included on the Canadian Domestic Substances list were extracted from PULSE. Substances were prioritized based on exposure characteristics and then hazard (persistence, bioaccumulation, toxicity). This prioritized 5 UVCBs with high tonnages and widespread use, that contained varied limitations in the breadth of available hazard data, highlighting areas that could be prioritized for data gap filling or in-depth hazard assessment. Overall, these case studies are conducted with Tier 0 data, thus require further investigation to confirm hypotheses. However, they serve to demonstrate how simple data available in the PULSE database can be used to highlight and fill data gaps, drive hypotheses, prioritize and augment existing hazard and risk assessments for UVCBs. Ultimately this work demonstrates the need for an expert-curated database containing detailed, publicly accessible information for UVCBs to advance the science for these complex substances.

4.04.T-02 Integrated Hazard Assessment of Permanent Aquatic Storage Strategy-Treated Fluid Tailings in a Canadian Pilot-scale Oil Sands Pit Lake

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Long-term management of fine tailings and oil sands process-affected water remains a significant challenge for reclamation in the Alberta oil sands (AOS) region of western Canada. Pit lakes are one option to address this

issue, provided they achieve chemical stability and support aquatic life. Lake Miwasin, constructed as a Permanent Aquatic Storage System (PASS), contains alum and polymer-treated fluid tailings. This study integrated multiple sediment toxicity assessments to assess the potential effects of the lake's bottom substrate on *Chironomus dilutus* larvae and characterize the hazards associated with polymer-treated tailings. Standard whole-sediment toxicity tests conducted in 2020 using deep-littoral and limnetic sediments showed toxicity in both zones, with survival ranging from 5 to 15%. A Phase I Sediment Toxicity Identification Evaluation demonstrated that ammonia and organic compounds likely contributed to the observed toxicity, particularly in the limnetic sediment. Polymer characterization and subsequent toxicity tests confirmed that the amphoteric polymer used during the PASS process did not contribute to toxicity to *C. dilutus* larvae, indicating that the polymer concentration in the expressed water was not a source of toxicity. Porewater tests conducted in 2021 and 2022 provided insight into spatial and temporal changes in sediment toxicity. The 2021 porewater test showed that the deep-littoral sediment porewater (0 to 3.33%) was more toxic than the limnetic porewater (16 to 53%). In contrast, the 2022 porewater test showed improved survival (>60%) in both zones, suggesting diminishing toxicity over time. An additional sediment depth horizon assessment was conducted in 2022 using limnetic sediment layers (0-2 cm versus bulk 0-10 cm) and in 2023 (0-2 cm, 2-5 cm, and bulk 0-10 cm). Generally, the results showed that the surficial sediment (0-5 cm) was less toxic than the bulk sediment in both years, indicating decreasing toxicity in the surficial sediment layer. *Pimephales promelas* early-life stage tests conducted with surface water samples collected in 2023 demonstrated that near-bottom water was toxic. EcoToxChip, a molecular tool for detecting changes in gene expression, will further determine the biological pathways of the complex mixture present in the lake water to help assess potential aqueous risk. Overall, these findings will help inform future sediment sampling and monitoring in this and similar pit lakes in the AOS region.

4.04.T-03 Environmental Risk Assessment of UVCBs: The Value of Multi-Generational Exposure Tests
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Substances of unknown or variable composition, complex reaction products and biological materials (UVCBs) pose unique challenges to regulators and product registrants, who are required to characterize their environmental fate and toxicity, specifically, it makes toxicity, environmental behaviour, risk, and classification evaluations arduous.

Due to the analytical challenges posed by UVCBs, ecotoxicity testing must be carefully designed to maximize the solubility and maintaining stable exposure concentrations. Achieving consistent concentrations in laboratory testing is complicated by degradation, volatility and adsorption to test vessels or organisms.

In the present study, we report results from a UVCB case study in which different aquatic toxicity tests were performed to evaluate reproductive endpoints: a standard chronic test on *Daphnia magna* and a non-commonly required life-cycle test on the benthic organism *Chironomus riparius*. Analytical checks of test concentrations in relevant matrices were performed to determine actual exposure.

In the chronic *D. magna* test, no substantial negative effects were recorded. Maintaining stable exposure concentrations during the test was challenging despite precautions such as solvent carriers and pre-conditioning of test vessels.

The life-cycle test with *C. riparius* revealed generational effects: an increase in fecundity at the highest concentrations in the first generation, followed by a decrease in emergence and altered sex ratio in the second generation. The test was performed by spiking sediment with a solvent carrier and adding food directly to the sediments in view of the high log K_{ow} of the UVCB. The concentrations decreased over the 58-day test period. However, the mass-balance analysis, including sediment, water and porewater compartments, allowed to confirm the UVCB affinity for sediment and to verify organisms' exposure.

Our results confirm the challenges associated with aquatic exposure assessment of UVCBs, particularly the difficulty of maintaining stable test concentrations. While no adverse effects were observed in the *Daphnia magna* reproduction test, the *Chironomus riparius* life-cycle test revealed generation-dependent effects at the highest concentrations. These findings highlight the importance of multigenerational sediment-based tests for identifying risks that may not be evident in standard aquatic toxicity assays.

4.04.P Environmental Hazard and Risk Assessment of UVCBs and Complex Mixtures: From Scientific Challenges to Regulatory Solutions

4.04.P-Th148 The Challenges of Aquatic Classification of Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs) and Complex Mixtures in Lubricant Additive Packages

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Lubricant additive packages have a wide range of applications in industrial, professional and consumer settings. These packages typically consist of a complex mixture of poorly water soluble, difficult to test mono-constituent, multi-constituent substances and Unknown or Variable composition, Complex reaction products or Biological materials (UVCBs). Whilst regulatory programmes such as the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) require aquatic toxicity data for determination of the hazard classification of the individual substances within these lubricant additive packages, there is no equivalent requirement for hazard classification of the whole mixture. However, Safety Data Sheets (SDSs) require hazard classification of the whole lubricant additive package. As such, lubricant additive packages are seldom classified based on the whole mixture, but instead by calculation from summation of the intrinsic hazards of all the individual substances in that mixture.

Many of the individual constituents of a lubricant additive package are considered toxic or very toxic to the aquatic environment, and in some cases, with long-lasting effects. However, many of these substances do not have a complete aquatic toxicity dataset; long-term aquatic toxicity data is rarely available. Therefore, chronic aquatic hazard classification is based on short-term aquatic toxicity data in accordance with Table 4.1.1(b)(iii) of the Global Harmonized System of Classification and Labelling of Chemicals (GHS). Furthermore, summation does not inform on how the individual constituents of a mixture can interact.

As such, there is an inherent uncertainty in the hazard classification of complex mixtures by summation approaches. Aquatic toxicity testing of lubricant additive packages, and classification based on these whole-mixture test data can provide a more robust approach to hazard classification. This poster will present several case studies of aquatic testing of lubricant additive packages, addressing the challenges of difficult-to-test substances, and provide a comparison of aquatic classification by both the summation and whole-mixture testing approaches. In many cases, data generated from mixture testing indicates lower hazardous classification compared to summation approaches, indicating the value of whole-mixture testing.

4.04.P-Th149 Integrating Spatial-Temporal-Extent Prioritization and a Tiered Mixture Framework: A Regulatory Strategy for Managing PPCPs in Korean Rivers

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The continuous release and pseudo-persistence of Pharmaceuticals and Personal Care Products (PPCPs) in aquatic environments pose significant ecological risks often underestimated by traditional single-compound assessments. This study established an integrated risk management framework by evaluating the spatiotemporal dynamics and mixture toxicity of 137 PPCPs across four major South Korean rivers (Han, Geum, Yeongsan, and Nakdong). A year-long LC-HRMS monitoring campaign detected 120 compounds. While ubiquitous compounds like metformin dominated in concentration (up to 42.7 µg/L), they posed negligible risks. Conversely, a conventional Risk Quotient (RQ) approach flagged broad ecological concerns but lacked discrimination for priority management. To address this, we applied the Spatial-Temporal-Extent (STE) prioritization methodology, integrating detection frequency and temporal persistence. This multidimensional approach refined the priority list, reclassifying specific stressors such as clotrimazole, fluoxetine, and olmesartan as primary ecological drivers warranting regulatory attention. Cumulative risks were assessed using a conservative Concentration Addition (CA) model. The Hazard Index (HI) and Risk Quotients based on the Sum of Toxic Units (RQ_STU) consistently exceeded safety thresholds, identifying fish as the most vulnerable trophic level (RQ_STU up to 24.2). Risks were significantly amplified during low-flow winter and spring periods, highlighting the influence of dilution capacity. Crucially, Maximum Cumulative Ratio (MCR) analysis categorized the majority of mixtures as Group I. This indicates that cumulative toxicity is predominantly driven by one or two high-potency chemicals rather than by the combined effect of many low-level substances. In conclusion, integrating STE prioritization with component-based mixture modeling offers a scientifically robust strategy superior to simple concentration-based monitoring. These findings provide essential evidence for establishing site-specific water quality criteria and targeted management policies for unregulated micropollutants.

4.04.P-Th150 A proposal for categorizing UVCBs for aquatic ecotoxicity testing

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Substances of ‘Unknown or Variable composition, Complex reaction products or Biological materials’ (UVCBs) are an increasingly important group of substances registered under the REACH regulation. These substances are complex mixtures that often do not have a fixed or well-defined chemical composition and consist of various constituents. UVCBs are considered “difficult-to-test substances” according to OECD 23 “Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures”. Standard aquatic ecotoxicity tests, developed for the testing of soluble substances, often prove to be not applicable for UVCBs as the individual constituents have different physico-chemical properties, which complicates the application to aqueous media. Furthermore, selective and sensitive analytical verification, and interpretation of measured exposure concentrations for these complex mixtures is difficult and lacks standardization. E.g., for UVCBs containing various constituents varying in their water solubility, an effect assessment can be difficult. Based on our practical experience on UVCB testing and to address these challenges, we propose a categorization system for UVCBs based on chemical similarity of constituents and based on difficulties for test application in aqueous media. This proposal focuses on complex chemical reaction products rather than biological materials which are all categorized as UVCB. In a first step, the substances are categorized into three categories according to their chemical complexity with regards to chemical structure and physico-chemical properties of the constituents. Following analytical considerations for method development and quantification strategies, the substance groups are further categorized by their applicability in aqueous test systems for reaching stable and uniform exposure concentrations. Eventually, different perspectives on data evaluation and discussion of analytical results and the ecotoxicological assessment are given for these subgroups. This categorization is intended to bring more clarity and uniformity in the performance and interpretation of ecotoxicological studies on UVCBs. Furthermore, it shall give guidance on whether testing of the whole substance or fractions/representative constituents of the UVCB may be reasonable. Eventually, these considerations may help regulators and other stakeholders with comparison between studies and overall evaluation of these studies within the REACH framework.

4.04.P-Th151 Organotin compounds in dust from Colombian: Concentrations, urban influence, environmental conditions, and socio-demographic factors

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Organotin compounds (OTs), widely used as stabilizers in PVC, catalysts, and biocides, represent a persistent class of semi-volatile indoor contaminants. Although their occurrence has been documented in household dust in Europe, North America, and Asia, data from tropical and Andean Latin American regions remain extremely limited. This study quantified monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT) in household dust from 30 homes in Cartagena, Medellín, and Bogotá, representing distinct climatic, geographic, and urban environments, as well as household socio-demographic tiers (low-, mid-, and high-income households). Phenyltins were also analyzed but remained below detection limits. Bogotá, a cold high-altitude Andean city (~2600 m) characterized by reduced natural ventilation and dense vertical housing, exhibited the highest concentrations of the sum of butyltins ΣBTs (667–2428 ng Sn/g). Cartagena, a coastal tropical city with high humidity and a mix of traditional and modern housing, presented intermediate levels (54–1140 ng Sn/g). Medellín, located in a temperate valley with moderate building density and favorable airflow, showed the lowest and most homogeneous concentrations (5–879 ng Sn/g). High-income households showed significantly higher ΣBTs, consistent with greater prevalence of PVC and synthetic laminated materials. MBT was the dominant compound, mirroring patterns reported in the USA and Europe. These findings highlight that indoor exposure to OTCs in tropical developing countries results from an interplay of urban form, climate, ventilation patterns, and material use, which extends beyond socioeconomic factors alone. This study establishes the first baseline for OTCs in Colombian indoor environments.

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4.04.P-Th152 UVCB Substances: Methodology for Substance Identification, Generation of Constituents and Selection of Representative Samples

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A thorough characterization of UVCBs (substances of Unknown or Variable composition, Complex reaction products, or Biological materials) has always been difficult due to the complex nature of their origin and/or unknown or variable composition. A well-defined substance identification is an essential precondition for a substance to satisfy the requirements of implementation of any chemical related legislation. The incomplete structural characterization also hinders an in silico hazard assessment, which role could become crucial for substance assessment when experimental data is not available. A methodology for definition of an unambiguous structural description was developed to address those issues. The methodology allows for the coding of constituents, their generation, calculation of important properties of UVCB constituents, and selection of representative samples. Two formats are developed: Generic Simplified Molecular-Input Line-Entry System (G SMILES) and Generic Graph (G GRAPH). G SMILES is a SMILES-based single line notation used for coding fixed and variable structural features of UVCBs. G GRAPH allows generation of constituents coded in G SMILES, calculation of physicochemical properties, simulation of reactions, selection of constituents and their storage in a text file. Two main types of selection of constituents are developed. The first one is an endpoint driven selection based on ranges of a defined physicochemical property, e.g., logKow values are critical when assessing acute aquatic toxicity. The second approach is a statistical selection of the minimum number of generated constituents which could be considered as representing the UVCB. This representative sample is selected in such a way that the structural variability and the properties of concern of the UVCB are estimated within a predefined tolerable error. The aim of the statistical selection is to enable the assessment of UVCB substances by reducing the number of constituents that need to be evaluated. The procedure has been applied successfully on theoretical examples and on real case studies.

4.04.P-Th153 Evaluation of the RTgill-W1 Cell Line Assay (OECD TG 249) for Predicting Acute Fish Toxicity of Surfactants

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Understanding short-term toxicity in fish is a key endpoint requirement in global regulatory frameworks and environmental risk assessment. Commonly this endpoint is fulfilled by the OECD TG 203 fish acute toxicity test. However, evolving legislation under the Chemical Strategy for Sustainability (CSS) attempts to reduce vertebrate animal testing. To achieve this ambition whilst ensuring environmental safety, robust, reliable, and regulatory-compliant alternatives are required. The RTgill-W1 Fish Cell Line Acute Toxicity assay (OECD TG 249) is proposed as an alternative. This assay has previously been shown to have good correlation with existing in vivo acute fish data for a variety of chemicals. However, to date only a limited number of surfactants have been tested using this assay and a clear applicability domain for this group of chemicals has not been definitively established. Surfactants often exist as multi-constituent or UVCBs (Substances of Unknown or Variable Composition) due to the variation in alkyl chain length and branched/linear structure. Their 'surface-active' nature, solubility, lipophilicity, and presence of ionizable headgroups also makes standard (or adapted) test methodologies challenging. Therefore, it is essential to evaluate the suitability of in vitro test designs for this chemical class. Here we present the results from an Environment and Health – Risk Assessment & Management (ERASM) Task Force, established to address these uncertainties. Initially, existing in vivo and in vitro experimental data (OECD TG 203, OECD TG 249) were collected from external sources for a range of nonionic, anionic, cationic, and amphoteric surfactants. Correlation analysis demonstrated strong agreement between in vivo and in vitro results, with the majority falling within one order of magnitude of the line of unity. Surfactants with high-quality compositional and in vivo data (specifically 2 nonionic, 2 cationic, 2 amphoteric,

4.04.P-Th154 Environmental Hazard Assessment of a UVCB under the REACH Regulation – Regulatory and Practical Perspectives

The environmental hazard assessment of UVCB substances poses significant challenges under regulations such as REACH. UVCBs are complex mixtures containing dozens to hundreds of constituents, often with unknown structures and variable composition. Current REACH guidance provides only limited direction for the analytical characterization required to ensure regulatory acceptance of ecotoxicity test results. Key challenges include verifying the UVCB analytically due to diverse physical-chemical properties of the constituents, such as solubility, degradation, and interactions with the test systems. An increased diversity in the properties of individual constituents makes it increasingly challenging to analyze representative constituents using a single analytical approach.

Hazard characterization of UVCBs is limited by technical feasibility, time, and cost. Regulatory and practical requirements frequently diverge, which necessitates case-by-case assessments for each substance. These challenges mean that registrants of UVCBs often face higher costs than those registering mono- or multi-constituent substances, resulting in an uneven playing field. Clear, harmonized regulatory criteria for selecting representative constituents and assessing technical feasibility are needed. Current guidance requires registrants to conduct extensive analyses before claiming that testing is technically not feasible, so efforts often resemble research rather than routine testing. A balanced approach between regulatory requirements and scientific considerations is essential to support UVCB registrants and restore fair competition.

Héloïse Thouément, Julie de Vriendt and Berend Mensink, Haskoning Nederland B.V., Netherlands

Essential oils contain multiple constituents, notably terpenes and terpenoids, with varying toxicities and behaviors. Concentrations range from trace level ($<0.1\%$) to major fractions ($>50\%$, some $>90\%$) of an essential oil. The composition of each essential oil batch varies, notably with plant growth conditions. Consequently, essential oils are considered as “unknown or variable compositions, complex reaction products or biological materials” (UVCBs). UVCBs pose challenges for environmental risk assessments as described in recent reviews.

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We illustrate differences in risk assessment results using a hypothetical citrus oil case-study, identifying the drivers of variability and assessing the applicability of the methods. This study will be relevant to the regulators as much as to the industry, as environmental risk assessments affect permissible production and use volumes under EU regulations. Comparing methodologies is particularly relevant with the current implementation of the One Substance, One Assessment approach.

4.04.P-Th156 Chemical Mapping of Naturals 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 consumers 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 naturals constituents, as to their Persistence, Bioaccumulation, Mobility and Toxicity potential and statistical analyses that will help understand and predict the fate and effects of naturals 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 naturals 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, 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 critically for the assessment of naturals 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.

4.04.P-Th157 Evaluating mitigation strategies to reduce chemical and biological pollutant transfer in biosolid-amended agricultural systems.

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The application of biosolids in agriculture provides nutrients and improves soil properties but can introduce emerging contaminants (ECs) and antibiotic resistance genes (ARGs), posing environmental risks. This study evaluated the fate of ECs, heavy metals, and ARGs in wheat cultivated in silty clay loam and sandy soils under five fertilization strategies: biosolids, biosolids amended with 10% biochar, biosolid-derived compost, inorganic fertilizer, and an unfertilized control. ECs were monitored at application, 30 days, and harvest (140 days), while ARGs were quantified in soil, straw, and seeds, and heavy metals were analyzed in soil. Biochar amendment accelerated EC degradation, reducing half-lives from 65 to 35 days and lowering ARGs abundance in soil and straw. Composting reduced the initial ECs and ARGs load but some compounds persisted longer in soils. Heavy metal concentrations remained below regulatory limits, and no ECs or ARGs were detected in seeds, indicating negligible transfer to edible tissues. These findings demonstrate that composting and biochar effectively mitigate chemical and biological contaminant persistence and mobility, enhancing the environmental safety of biosolid application in agricultural systems.

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4.04.P-Th158 Tracking alkylated decalins during biodegradation of a complex hydrocarbon substance to derive primary half-life data

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Assessing the biodegradability of complex hydrocarbon substances for chemical registration—such as under the REACH regulation—requires evaluating hundreds to thousands of individual constituent chemicals. A practical alternative to testing multiple individual constituents is to test a whole complex hydrocarbon substance and derive the primary biodegradation half-lives of individual constituents by tracking the observed decay of signal peaks in GCxGC-FID data obtained from successively biodegraded samples, as previously demonstrated by the authors. In this work, the authors focused on generating primary biodegradation half-life data for one representative group of hydrocarbons: the alkylated decalins. The eventual goal is to determine how structural features, particularly branching, influence primary biodegradation rates.

In previous work, GCxGC-FID data provided limited information about the chemical identities of the separated constituents, and these studies encountered challenges when attempting to associate the GCxGC-FID peaks with corresponding GCxGC-TOFMS peaks that could be further identified by Non-Target Analysis (NTA) techniques. For several of the studied constituents, chemical identity information from GCxGC-TOFMS data could not be clearly attributed to the calculated primary biodegradation rates from GCxGC-FID data. To address these limitations, we explored methodological adaptations to the analytical and data processing approaches, including:

1. Analyzing a series of successively biodegraded samples by reversed-phase GCxGC-TOFMS, including a prefractionation step to remove the aromatic fraction
2. Tracking the decay of the quantified peak signal of selected ions diagnostic of alkylated decalins in the GCxGC-TOFMS data obtained from the successively biodegraded samples
3. Using existing Non-Target Analysis work-flows on the co-located TOFMS mass spectrum to identify chemical structure information for each selected ion peak that was tracked. We also consider the GCxGC retention time relative to other alkylated decalin peaks.

This improved workflow would enable the targeted identification and characterization of alkylated decalin structures in a straight-run gas oil sample that had been previously tested.

4.04.P-Th159 Defining aquatic toxicity endpoints for pesticide mixtures – Why the “cat must chase its own tail”

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Detailed guidance on deriving aquatic toxicity endpoints for pesticidal products containing multiple active substances or toxicologically relevant co-formulants is provided by EFSA (2019). However, feedback from Competent Regulatory Authorities on the acceptability of endpoints reported in studies has been inconsistent, suggesting that the current guidance leaves some room for interpretation.

While such variability may be undesirable when seeking universally accepted approaches, a certain degree of flexibility can be useful to support scientifically robust data interpretation. The EFSA framework adopts a conservative stance by defining toxicity solely on the basis of analytical recovery in the test matrix, but without accounting for the toxicity of individual components. This method yields meaningful endpoints only when all critical components are similarly persistent and toxic. Conversely, it may overestimate actual toxicity if a component with low stability and limited toxicological relevance is present.

Greater scientific rigor is therefore needed to derive meaningful endpoints for combination products, ensuring risk assessments are protective without being excessively conservative. In environmental risk assessment, measured product toxicity is also used to evaluate the predictive value of modelled mixture toxicity using Concentration Addition (CA), including the derivation of Model Deviation Ratios and the identification of potential synergistic effects that may occur due to the combined exposure to multiple constituents present in the formulation. Although measured product toxicity is valuable for detecting synergisms, careful judgment should be applied in its calculation.

While empirical toxicity data inform on the applicability of CA, CA together with the identification of toxicity drivers should also be employed to scrutinize the way aquatic endpoints are defined, emphasising the circular nature of this reasoning while maintaining a sense of proportion to ensure meaningful risk assessments for aquatic organisms.

4.04.P-Th160 Dioxins in Food and Feed: Update of the 2018 EFSA Risk Assessment

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The European Food Safety Authority (EFSA) was established in January 2002 as an independent body providing scientific advice and communication on risks associated with the food chain (Regulation (EC) No 178/2002).

Polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) do still occur in the environment and may enter the food-chain and as a result accumulate in humans. In 2018, the EFSA Panel on Contaminants in the Food Chain published an Opinion on the health risks for humans and animals related to the presence of PCDD/Fs and DL-PCBs in food and feed. A Tolerable Weekly Intake (TWI) of 2 pg Toxic Equivalents (TEQ)/kg bw per week was derived for humans, expressed using the Toxicity Equivalency Factors set by WHO in 2005. Average and high exposures were, respectively, up to five and 15 times the new TWI in adolescents, adults and the elderly, while toddlers and children up to 10 years of age had a similar range of exceedances of the TWI. The EFSA Opinion recommended, among others to re-evaluate the 2005 WHO-TEFs in order to take into account new in vivo and in vitro data (in particular, more insight into the reported lower relative potency of PCB-126 in humans).

In 2024, WHO published its updated WHO-TEF values. Following this publication, the European Commission requested EFSA for an update of the scientific Opinion on the risk for animal and human health related to the presence of PCDD/Fs and DL-PCBs in feed and food on the basis of the new 2022 WHO-TEF values.

In addition to the existing data, all relevant toxicological information in experimental animals and humans published since the 2018 Opinion was taken into account for the hazard and risk characterisation. An update of the dietary exposure assessment was also performed considering the occurrence data on PCDD/F and/or DL-PCBs in food submitted to EFSA between 2013 and 2023. The draft updated scientific Opinion underwent public consultation from November 2025 until January 2026.

All aspects of the risk assessment where the new 2022 WHO-TEFs would have an impact will be presented. These include the hazard characterisation, e.g. the derivation of the health-based guidance value (Tolerable Weekly Intake (TWI)), as well as the changes in occurrence in food and dietary exposure assessment expressed in TEQ following the use of the 2022 WHO-TEFs compared to the use of the 2005 WHO-TEFs.

4.04.P-Th161 High-Throughput Assessment of Predicted Hydrocarbon Degradation Products Using Integrated QSAR Workflows and Applicability Domain Analysis

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Petroleum substances, particularly those classified as substances of unknown or variable composition, complex reaction products or biological materials (UVCBs), present a significant challenge for environmental hazard assessment due to their complex and variable chemical profiles. One requirement under the European REACH and CLP chemicals regulations is the identification and evaluation of the persistence, bioaccumulation, mobility, and toxicity (PBMT) of their expected degradation products, which are often more structurally diverse than the parent compounds, including more polar and potentially charged compounds.

To address this, we developed a high-throughput computational workflow to assess over 6.45 million predicted degradation products of >127,000 hypothetical parent structures. To manage datasets of this magnitude, the team developed a high-throughput, reproducible data-processing architecture using Polars as the central analytical engine. The design emphasizes scalability, traceability, and regulatory compliance within a unified QSAR/QSPR framework. We integrated multiple QSAR models, including OPERA, to predict PBMT endpoints, and evaluated the applicability domains of each model and identified gaps where polar or charged metabolites fall outside existing QSAR coverage.

Preliminary results generated with a subset of the data show that a substantial proportion of metabolites (approximately 33%) are outside the applicability domains of commonly used OPERA models, especially for bioaccumulation and mobility endpoints. A subset of compounds was not covered by any model, indicating the potential need for targeted experimental testing. This work demonstrates the importance of combining multiple QSARs and applicability domain analyses to ensure robust hazard assessment of complex petroleum-derived substances and their transformation products.

Our findings support the development of more inclusive and scalable computational tools for regulatory

chemical assessment and provide a framework for identifying data gaps in UVCB metabolite evaluation. By enabling high-throughput screening of structurally-diverse hydrocarbons (including polar/ionised species) in a reproducible data-engineering environment, the platform contributes to the field of environmental chemistry, hazard and exposure assessment, facilitating modelling of chemical behaviour across compartments, monitoring-modelling integration and evidence-based decision support.

4.04.P-Th162 MEED: Finalization of the Multiyear Metals Environmental Exposure Data Collection program Anticipating the Challenges of the EU Zero Pollution Ambition Policy and the Combined Exposure to Mixtures

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The Chemicals Strategy for Sustainability (CSS) is a key pillar of the EU Zero Pollution Ambition (ZepA), and a building block of the Green Deal. This policy aims for reaching good quality status of the environment by achieving exposure levels of chemicals that are no longer being harmful to health and the environment. This challenges environmental and chemicals regulations given focused on substance specific risk assessments and limits. The effects of combined exposure of mixtures and ensuring they do not affect biodiversity are therefore new areas of concern in regulatory risk assessments. The introduction of a Mixture Allocation Factor (MAF) to demonstrate safe use of chemicals and lack of impact of mixtures on ecosystems, is a default approach considered by regulators. This poses a significant scientific challenge particularly for metals, given their natural occurrence and broad and growing use in Green Deal applications such as electric vehicle batteries, solar cells, ... This complex challenge drove the sector to conduct a comprehensive “Metals Environmental Exposure Data” (MEED) gathering programme, including the development of scientific concepts and targeted test work to assess combined mixture exposure. MEED ran for 4 years, built on 6 interlinked projects. The first was the identification of metals that contribute the most to the combined risks to the environment, followed by an extensive investigation of today’s regional background levels of metals, and an update on the STP emissions contributions as a key source of consumer input. A review and reappraisal of existing knowledge on metals mixtures and metals-organics mixtures interactions allowed to define data gaps and to perform gap-filling research for their toxicity and interactions. Next, this evidence was used in a tiered assessment system for local and regional biodiversity impact assessment, including biomonitoring and environmental DNA screening for specific organism groups. Finally, the outcomes were used to design a science-based concept to improve the assessment of combined effects for regulatory schemes like REACH. This poster presents the overall process, main outcomes and conclusions of MEED, while detailed assessment are presented in complementary posters and presentations. MEED delivered a large amount of data and relevant concepts for use in chemicals registration and environmental permit updates, key to assess progress with the EU ZepA policies in a scientific way.

4.04.P-Th163 Assessment of Structural and Functional Changes to the Microbiome of Aquatic Microcosms Caused by Heavy Metals in E-waste: Cerium and Lanthanum

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Technological development is increasing the consumption, use and subsequent disposal of electronic objects, generating a new and worrying type of waste known as e-waste. It is estimated that e-waste production will reach 74 million tonnes by 2030. This presents a significant global environmental problem, as many of these products contain toxic metals that can leach into aquatic ecosystems and cause environmental and health problems. This study aims to determine the impact of the various metals present in e-waste on aquatic ecosystems by evaluating changes in microbial populations in both biotic and abiotic matrices. To this end, artificial microcosms containing sediment and water, along with the bioindicator organism *Scrobicularia plana*, have been designed and placed under controlled conditions. The effects of the metals cerium (Ce) and lanthanum (La) were studied both individually and as a mixture (Ce+La). The microbiome of the sediment and digestive gland of *S. plana* was identified by isolating genomic DNA from these matrices and sequencing their 16S rDNA. The most abundant phyla in the sediments were Proteobacteria, Actinobacteria, and Bacteroidetes, whereas the most abundant phyla in the digestive gland of *S. plana* were Tenericutes, Proteobacteria, and

Firmicutes. At a lower taxonomic level, the Ectothiorhodospiraceae family was most abundant in the sediments, whereas the Mycoplasmataceae family was most abundant in the digestive gland of *S. plana*. All results were statistically evaluated. Additionally, alpha and beta diversity, as well as microbiome functionality using the FAPROTAX database, were analysed. The results obtained reveal the effects of metals on microbiome structure and its consequences. Our ultimate goal is to identify future exposure biomarkers for these contaminants.

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4.04.P-Th164 Establishing a Robust Boundary Composition taking the Example of a Complex UVCB Substance: Analytical and Regulatory Challenges

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Accurate substance identification is a central requirement under REACH. However, UVCB substances often lack robust, standardized analytical frameworks to define their boundary composition, which summarizes and comprises the composition in set boundaries. A representative UVCB substance was selected to illustrate the challenges associated with deriving a robust boundary composition. The complex mixture is the reaction product of a condensation reaction resulting in a wide range of molecules from volatile constituents with low molecular weight to oligomers with high molecular weight. Because of this inherent variability, defining a reliable boundary composition is essential to ensure regulatory compliance and to support a scientifically sound Substance Identity Profile (SIP). According to the European Chemicals Agency (ECHA) guidance “Identification and naming of substances under REACH and CLP”, all relevant constituents—regardless of their abundance—must be identified and quantified down to the respective reporting thresholds, particularly when they may influence classification or PBT assessment. Inconsistent or incomplete analytical data, such as reporting vague concentration ranges, undermines regulatory acceptability and the scientific integrity of the SIP. For such complex UVCB substances, conventional material balance approaches are not directly applicable due to the inherently diverse composition. This work outlines the analytical challenges encountered and presents a multitechnique strategy developed to achieve reliable quantitative characterization, while relying on a practical, low-effort analytical workflow that allows data to be obtained with minimal resource expenditure. The integrated approach combines LC-UV fingerprinting as key element, sulfated ash determination, and Karl Fischer water analysis, supplemented when needed by LCMS and gel permeation chromatography (GPC). Together, these methods provide a reproducible, sensitive, and comprehensive analytical dataset across laboratories.

We describe the data evaluation concept enabling consistent quantification of relevant constituents and discuss the regulatory context in which these analytical requirements are embedded. The proposed methodology strengthens characterization standards for UVCB substances and offers a transferable framework for addressing similar complexities in boundary composition definition and SIP development.

4.05 Environmental Risks of (New) Chemicals Used in Maritime Transport

4.05.T-01 Bringing circularity and sustainability into the maritime antifouling coatings industry by reusing dead batteries

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Alkaline batteries are used in various electronic devices and when they are discarded, they pose a threat to the environment and human health. ZnO is a semiconductor with a band gap of 3.37 eV and a high excitonic binding energy of 60 meV. These properties give it important technological applications, among them, ZnO has antimicrobial and antifouling activity that can be used to bioactive coating formulations. Marine biofouling on ship hulls decreases speed and increases fuel consumption and greenhouse gas emissions. The present study aims to propose a method to revalorize the Zn present in the alkaline batteries by developing an active nanoform, incorporating it in coatings, and assessing its anti-fouling efficacy and ecotoxicity. Briefly, zinc was

recovered through an acid leaching process using biogenerated H_2SO_4 ($\text{H}_2\text{SO}_4\text{b}$). Once the Zn^{2+} ions were dissolved, a Na_2CO_3 solution was added. The resulting precipitate was dried and separated into two fractions: one was calcined at 500 °C for 2 hours (C-ZnO), and the other, the uncalcined fraction (WC-Zn), was doped by wet impregnation with H_2SO_4 , followed by calcination, and designated WC-ZnO/S. The solids were characterized by ICP-OES, XRD, SEM, and TEM. Three self-cleaning antifouling paints (P) were formulated with a 7% concentration of a biocidal additive: P_C-ZnO, and P_WC-ZnO/S plus a biocide-free control (P_CTL). The antibiofilm and the short-term ecotoxicity of the coatings with the additives were evaluated. Quantitative determination of the components present in C-ZnO and WC-ZnO/S using ICP showed that zinc (Zn) is the major element, along with sulfur (S), sodium (Na), and iron (Fe). The predominant phase of the solids was zincite (ZnO). SEM analysis showed morphological differences between the two solids: C-ZnO exhibited a nanorose-like shape, while WC-ZnO/S had a star-like shape. TEM analysis revealed particle sizes greater than 20 nm for C-ZnO and 50 nm for WC-ZnO/S. Bioassays suggest that the studied solids are potential antifouling agents and that their incorporation into paints would help prevent the adhesion of microorganisms. In this context, zinc recovery and the manufacture of new materials with potential applications as environmentally friendly antifouling additives represent significant waste valorization with clear environmental benefits by fostering circularity and sustainability, particularly in the maritime industry.

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4.05.T-02 Ecotoxicological and Biodegradability Assessment of Antifouling Paints for Marine Applications (PROJECT - NAUTILUS)

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Biofouling negatively affects marine infrastructures and leads to substantial economic costs. Conventional antifouling paints used on boats contribute to marine pollution by releasing microplastics and toxic biocides, potentially harming marine species. Additionally, fossil-based paints increase greenhouse gas emissions and deplete finite resources. The NAUTILUS project, funded by the Spanish Ministry of Science and Innovation, aims to develop safer and biodegradable alternatives by designing new biobased and biodegradable antifouling paints.

Four biobased formulations based on biodegradable polymers such as PHA and PLA were developed without biocides to reduce their ecotoxicity potential. A tiered assessment was conducted, including ecotoxicity and biodegradability testing, to select the most promising alternatives. The same approach was applied to seven acrylic-based commercial paints to identify a commercial benchmark for comparison.

Physicochemical tests using FTIR, TGA, and ICP-MS evaluated paint degradation and substance release during seawater exposure. Copper-based commercial paint showed high Cu release (0.29 mg/L) and DCOIT-based paint released 39.3 mg/L, leading to their exclusion as safe options. Based on degradation and surface roughness results, a biobased, biocide-free PHA paint was selected.

Biodegradability assessments following OECD 301F confirmed one acrylic-based biocide-free paint as readily biodegradable (>60% in freshwater after 28 days). Under ISO 18830 marine conditions, two commercial paints and the PHA-based paint achieved >60% biodegradation after 120 days, meeting the standard criteria.

Ecotoxicity testing across multiple marine species revealed that paints containing DCOIT had the highest toxicity, with effects observed in algae, crustaceans, and bioluminescent bacteria. Both acrylic and PHA polymers were evaluated to confirm their safety. The biocide-free PHA paint demonstrated lower ecotoxicological impact compared to traditional commercial paints containing biocides.

In conclusion, the NAUTILUS project successfully developed a biobased antifouling paint using a PHA matrix. The new formulation is biodegradable and does not cause significant ecotoxic effects on tested marine organisms. Ongoing physicochemical research aims to further understand the substances released during the

paint's lifespan in seawater, supporting its potential as a sustainable alternative to conventional antifouling coatings.

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4.05.T-03 Environmental Safety Assessment of Liquid Organic Hydrogen Carriers for Energy Transport: Towards Benign-by-Design Solutions

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The maritime industry's transition from fossil fuels to renewable energy faces significant challenges. While wind and solar power are being developed, their intermittent nature and geographical limitations necessitate efficient energy storage solutions. Hydrogen has emerged as a promising energy carrier, with multiple storage approaches available. Liquid Organic Hydrogen Carriers (LOHCs) offer advantages over compressed (CGH₂) and liquefied (LH₂) hydrogen by eliminating high-pressure or cryogenic storage requirements. However, LOHCs require energy-intensive hydrogenation/dehydrogenation processes and present potential environmental hazards that remain poorly characterized.

As LOHC technology evolves, multiple carrier molecules are being evaluated for maritime applications, creating opportunities to select environmentally acceptable options alongside technologically efficient ones. This study provides a comprehensive preliminary environmental hazard assessment of 24 LOHC carrier systems across four key categories: persistence, bioaccumulation potential, aquatic toxicity, and mobility. Environmental hazard profiles were systematically evaluated for each LOHC system in both hydrogen-lean and hydrogen-rich forms. Results revealed that most LOHC systems exhibit at least one form that is either bioaccumulative or mobile in aquatic environments. Critically, the hydrogen-rich form typically demonstrates poor biodegradability, identifying biodegradation potential as a key design parameter for developing new LOHC carriers. Structure-Activity Relationships (SARs) were developed from the compiled dataset, enabling identification of molecular features associated with specific hazards. Common hazard patterns, critical knowledge gaps, and recurring shortcomings across LOHC systems were systematically characterized. Two oxygen-containing LOHC systems are identified as promising low-hazard alternatives. This proactive environmental assessment framework supports informed carrier selection, enabling the maritime industry to pursue energy transition goals while minimizing ecological risks associated with emerging hydrogen storage technologies.

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4.05.T-04 Ammonia as a Maritime Fuel: A Review of Environmental Risk Dynamics during Bunkering and Shipping Spills

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Ammonia is a promising carbon-free maritime fuel because of its high energy-to-volume ratio. However, it also poses risks to human health and marine ecosystems, hampering its regulatory and social acceptance. In the absence of robust data on high-volume ammonia spill risks, this review evaluates the existing knowledge of marine-related hazards unique to large- and small-scale accidental spills. The main challenge is to understand how different forms of ammonia, release conditions, environmental processes, and mitigation strategies combine to determine the overall risk in marine spaces. To address this challenge, evidence is synthesised from its historical application as an industrial chemical, theoretical studies on large-scale releases, documented physical and chemical behaviour, and ecological impact. Ammonia has primary hazards, including toxicity, flammability,

and explosiveness, and secondary hazards arise from combustion byproducts and biological transformation. Storage and wind under general atmospheric conditions strongly influence spill behaviour. The fully refrigerated ammonia form has reduced airborne dispersion, thereby enhancing human safety. This property increases the likelihood of high-concentration contamination in water, intensifying local ecological impacts. Wind speed disperses ammonia, reducing the local risk, but can occasionally intensify the risk to distant areas under light wind speeds and stable atmospheric conditions. Spills can cause acute toxicity and disrupt the natural biological pathways that convert ammonia into less harmful nitrogen forms. Under limited dilution, transformative products such as nitrites can reach toxic levels. Non-toxic ammonia, nitrite, and nitrate levels can stimulate harmful algal blooms. Regarding mitigation-related insights and risks, spills are expected to result from human error or equipment failure. Existing mitigating strategies for airborne ammonia may occasionally lead to conflict between human and ecological safety, highlighting the need to reduce overall risks through improved operational procedures, engineering design, ammonia-absorption technologies, and integrated approaches to hazard management. Overall, the physical, biological, and operational components of ammonia must be integrated for risk evaluation, as this will support the development of effective regulatory measures and mitigation strategies as ammonia becomes increasingly important in maritime energy systems.

4.05.T-05 Nature-based Antifouling Strategies Using *Posidonia oceanica* Leaf Extracts in Silicone Coatings for Marine Aquaculture

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In aquaculture, various approaches have been used to address biofouling, including mechanical net cleaning, biological control, and chemical antifoulants. However, chemical antifoulants may release organic and/or toxic solvent-based contaminants into the environment. Therefore, sustainable aquaculture promotes the use of antifouling polymers that incorporate naturally derived bioactive compounds in netting materials. *Posidonia oceanica*, a Mediterranean seagrass, is rich in primary and secondary metabolites such as terpenes, flavonoids, and phenolic compounds. It has demonstrated strong antifouling performance among its applications in human activities. Alcoholic extracts of *P. oceanica* leaves were prepared and added to a silicone-based polymeric matrix (PDMS) at concentrations of 0, 90, 135, and 180 mg L⁻¹. These composite materials were applied to 5 × 5 cm Dyneema® net samples using dip-coating. To test their efficacy, all samples, including untreated Dyneema® nets as controls, were mounted on a rigid frame and immersed in seawater at Livorno touristic Harbor (Italy). The nets were monitored every two weeks for 14 weeks, from early March to late June 2025, with photographic documentation. At the end of the exposure, efficacy was assessed by cleaning the samples with a high-pressure water jet (150 bar, 50 cm distance) for 10, 20, 60, and 90 seconds. Photographic data were analyzed using ImageJ software to quantify fouling coverage, mesh occlusion, and cleaning efficiency. The results showed that nets treated with PDMS-based coatings were more effective than controls in reducing surface coverage, occlusion, and improving cleaning efficiency. Of all treated samples, the coating with 180 mg/L *P. oceanica* extract was the most effective across all parameters. This improvement was particularly evident up to the sixth week, with significant reductions in fouling coverage and mesh occlusion compared to all other treated nets, and higher cleaning performance even after 90 seconds of water-jet exposure. These findings highlight the potential of *P. oceanica* leaf extracts as bioactive additives in silicone coatings. This approach provides a nature-based antifouling strategy for sustainable aquaculture and offers opportunities for further study through laboratory tests.

4.05.P Environmental Risks of (New) Chemicals Used in Maritime Transport

4.05.P-We324 Ecotoxicity and circular revalorization of Bio-Sourced and Thermosetting Polymeric Coatings

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Protective coatings are among the most widely used and industrially relevant coatings, and within this category, those based on synthetic polymers are a key class of materials that can be broadly divided into thermoplastic and thermosetting materials. However, coatings, especially the thermosetting ones used in high-performance applications, pose significant environmental concerns. Given the unlikely elimination of polymers from our

society, it is crucial to better understand the environmental risks associated with coatings and to develop strategies to revalorize and upcycle end-of-life polymeric materials, which are currently treated as non-added-value waste. Therefore, this work aims to present a holistic framework along with the very first results of the assessment of the environmental hazard of innovative bio-sourced coatings (alkyd-based) and conventional thermosetting coatings (polyurethane-based). This assessment involves standard ecotoxicological assays exposing eight different marine species to the compounds' leachates over time, evaluating endpoints such as growth inhibition, embryotoxicity, air survival, condition index, and biochemical biomarkers. The revalorization potential of end-of-life polymeric materials will also be evaluated through additive manufacturing using degraded materials in the matrix to produce 3D printing filaments, as well as through bioconversion methods based on the capacity of yellow mealworms (larvae of *Tenebrio molitor*) to digest these materials. This includes assessing their survival, growth and bioconversion rates to create novel add-value eco-products, contributing to the reduction of coatings' environmental footprint and a "zero" pollution environment.

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4.05.P-We325 Are Innovative Ultra-Low Friction Silicone Foul-Release Coatings Viable Green Alternatives?

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The colonization and adhesion of fouling organisms to submerged metal infrastructure pose a persistent challenge for the global maritime sector, with profound economic and environmental implications. Conventional approaches, centered on biocidal antifouling coatings, have been adopted to mitigate this phenomenon; however, their toxic impact has not been sufficiently explored. The main active components of these polymeric antifouling coatings, such as organic or metallic biocides, demonstrate toxicity to several non-target species. In this context, silicone-based foul-release coatings (FRCs) emerge as an alternative due to the absence of biocides in their formulation and the surface characteristics that hinder adhesion or facilitate the removal of organisms. The present study aims to evaluate the ecotoxicity of leachates from four commercial FRC coatings from the company GIT (XGIT-FUEL Black, XGIT-FUEL Blue, XGIT-FUEL Red, and XGIT-FUEL White), comparing them with a commercial paint containing copper. The coatings were applied to microscope glass slides (n=3 for each coating) and immersed in aerated artificial seawater for up to one year, with periodic collection of water aliquots containing the coatings' leachates for physical-chemical analysis and ecotoxicological testing. To assess ecotoxicity, three species of microalgae were tested (*Chaetoceros calcitrans*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii*; ISO 10253), a rotifer (*Brachionus plicatilis*; ISO 19820), and a crustacean (*Artemia salina*; ASTM E1440-91). The results showed that globally the tested FRC coatings did not have a significant effects on the four species tested over time (when significant effects were detected, they were limited to a maximum of +20% relative to the negative control). However, the commercial self-polishing coating (SPC) containing copper caused acute toxicity in *Brachionus plicatilis* after just one day of immersion, associated with the release of a high amount of copper. Overall, these results suggest that the FRCs currently marketed by GIT are promising alternatives, with a lower environmental impact than the most commercially successful category of antifouling coatings /Cu-based SPC) worldwide.

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4.05.P-We326 Biodiesel Produced in Brazil as Candidate Low-Carbon Fuel for Maritime Transportation under the International Maritime Organization Strategy on Reduction of Greenhouse Emissions from Ships

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The International Maritime Organization (IMO) has launched a strategy to reduce greenhouse gas (GHG) emissions from ships, which account for 3% of global emissions, in support of the UN Sustainable Development Goal 13 (take urgent action to combat climate change and its impacts). Projections indicate that 64% of the reduction by 2050 will be achieved using low-carbon fuels, such as biodiesel, of which Brazil is the world's third-largest producer, with 9.1 billion liters produced by 58 plants in 2024. Its origin from renewable biomass and residues, combined with significantly lower GHG emissions compared with conventional fuels, makes biodiesel an important contributor to the country's energy matrix and a valuable candidate low-carbon fuel for maritime transportation.

Our study assessed the GHG emissions of biodiesel from soybean and beef tallow, which account for 72.4% and 6.4% of the feedstock used in Brazil in 2024, respectively, following the methodological rules set in the IMO Life Cycle Assessment Guidelines. The scope included the biofuel production to the end-use (Well-to-Wake or WtW), which is the combination of scopes Well-to-Tank (WtT) (from primary production to the transportation of the fuel to the ship's tank) and Tank-to-Wake (TtW) (from the ship's fuel tank to the exhaust). For soybean biodiesel, the WtT scope encompasses soybean production, the extraction of vegetal oil and its conversion to biodiesel, and the transportation stages. For tallow biodiesel, in addition to the conversion to biodiesel, the industrial stage includes the treatment of tallow, a by-product of the slaughterhouse.

Results indicate GHG emissions for biodiesel of 9.78 (WtT for tallow), and 23.84 (WtT for soybean, not including land use change), plus 4.18 g CO₂e/MJ (TtW for both feedstocks). We found that fossil CO₂, from methanol used during the transesterification process in all biodiesel plants in Brazil, released during fuel combustion on the ship (TtW), has a significant impact of 3.74 g CO₂e/MJ. Using ethanol instead of methanol could reduce the impact to 0.48 (TtW). Overall, biodiesel compares favorably with heavy fuel oil, a conventional maritime fuel with emissions of 91.60 g CO₂e/MJ (WtW).

Biodiesel, which is a well-established biofuel in Brazil with a capacity capable of providing a large increase in production, can be a valuable piece of the IMO's strategy to reduce the GHG emissions from ships globally.

4.05.P-We327 Environmental Risk Assessment of 'Green' Fuels in shipping

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International shipping is currently undergoing a profound transformation driven by the global ambition to achieve climate neutrality. Central to this transition is the replacement of conventional fossil fuels with alternative energy to fossil fuels often referred to as 'green' fuels. While the climate benefits of adopting green fuels are evident, particularly in terms of lowering CO₂ emissions to the atmosphere, the broader environmental implications remain insufficiently understood. Unlike conventional fossil marine fuels, which have well-documented spill scenarios and established response strategies, green fuels present novel challenges and a need for a systematic assessment of environmental risks associated with accidental spills, operational discharges, and long-term exposure in marine ecosystems.

Our research addresses this knowledge gap by conducting a comprehensive environmental risk assessment of spill and operational discharges of the green fuels ammonia and methanol intended for maritime use. The present assessment integrates literature values on chemical property analysis, and an ecotoxicological evaluation, with a comprehensive scenario-based modeling to estimate dispersion and concentrations in seawater and air under different spill conditions and localities in Danish waters. By simulating worst-case scenarios and operational leakage/discharges events, we aim to identify critical thresholds for toxic effects on marine organism and seabirds and inform the development of mitigation strategies.

4.05.P-We328 Effects of Ammonia Fuel Spills on Marine Microorganisms: Initial Insights from a Mesocosm Experiment.

Julie Svendsgaard, Janne Fritt-Rasmussen, Kim Gustavson, Christian Juncher Jørgensen, Hans Jakobsen and Sofie Amalie Sønderby Rask, Aarhus University, Denmark

Ammonia (NH₃), projected to become a dominant marine fuel by 2050, is a well-known inorganic nitrogen compound with documented toxicity to aquatic organisms. However, knowledge of its behaviour, ecological effects and potential cumulative impacts under high-concentration spill scenarios remains limited. Because microorganisms play a key role in biogeochemical cycling, understanding their response to NH₃ spills is critical for assessing ecosystem-level impacts.

We conducted a 14-day in situ mesocosm experiment in a Danish fjord (summer 2025), exposing natural microbial assemblages (<200 µm) to seven pulse additions of NH₃ across a concentration gradient (2–200 mg NH₄OH-N/L). Changes in phytoplankton and heterotrophic bacterial populations were assessed using flow cytometry, while nutrient speciation, pH, and oxygen dynamics were monitored. Preliminary results indicate that concentrations >20 mg NH₄OH-N/L suppressed phytoplankton growth throughout the experiment, with phytoplankton fluorescence recovery observed at 20 mg NH₄OH-N/L after 12 days. During the first week, growth inhibition occurred at 4–9 mg NH₄OH-N/L.

Our preliminary findings suggest that NH₃ spills at the tested concentrations may pose lethal risks to lower trophic levels in coastal marine ecosystems. Pending our chemical and community composition data, we expect to gain further insights into exposure thresholds and community recovery dynamics. Ultimately, our goal is to quantify the ecotoxicity of NH₃ spills at phytoplankton and bacterial population level and assess their implications for coastal marine ecosystem functioning.

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4.06 Filling the Gap: Solutions for Monitoring, Modelling and Assessment of Chemical Pollution Under Data Scarcity and Low-Capacity Conditions

4.06.T-01 Fuelwood Consumption Patterns and Spatial Distribution of PM_{2.5} and PM₁₀ Emissions in Thulamela Municipality, Limpopo Province, South Africa.

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Fuelwood remains a dominant household energy source in Thulamela Municipality, contributing significantly to fine particulate pollution and associated health risks. This study examines household fuelwood consumption patterns and maps the spatial distribution of PM_{2.5} and PM₁₀ emissions resulting from residential wood combustion (RWC). Fuelwood activity data were obtained through scale-based measurements from households in Gondeni and Lunungwi villages. A bottom-up emission estimation approach was applied, integrating seasonal activity data with established emission factors, and allocating emissions across the municipality at 1-km resolution. The results show clear seasonal variation in daily fuelwood use, with households consuming 7.27 kg/day in summer, 10.33 kg/day in autumn, 12.92 kg/day in winter, and 7.61 kg/day in spring. Winter exhibited the highest levels of both wood consumption and particulate emissions. Average annual household emissions were estimated at 5.34×10^{22} t/year for PM_{2.5} and 6.04×10^{22} t/year for PM₁₀. Spatial analysis revealed distinct emission hotspots in the municipality's southwest and northwest, aligning with densely populated settlements that rely heavily on fuelwood. This study provides the first spatially resolved baseline for PM_{2.5} and PM₁₀ emissions in Thulamela Municipality, South Africa, related to the RWC. The findings fill a critical data gap in rural household energy emissions and offer a valuable decision-support tool for clean-cooking initiatives, targeted air-quality management, and future exposure-risk modelling in low-income communities.

Keywords: Residential wood combustion (RWC); PM_{2.5} and PM₁₀ emissions; Fuelwood consumption; Spatial distribution; Bottom-up emission approach; Thulamela Municipality; South Africa; Air-quality management.

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4.06.T-02 Toxicity assessment of sediments under the influence of submarine outfalls along the coast of São Paulo, Brazil.

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Combining toxicity and genotoxicity tests, along with chemical analysis, is a solid strategy to assess the quality of sediment samples affected by submarine outfalls. This study aimed to evaluate the quality of sediment samples collected in areas impacted by these discharges along the São Paulo coast in Brazil. Acute and chronic tests were conducted using the tropical epibenthic organism *Parhyale hawaiensis*, as well as the *Salmonella*/microsome assay. Non-target chemical analysis was also performed. Sediment samples were collected from areas subjected to three wastewater submarine outfalls (Praia Grande, Praia das Cigarras, and Santos). Mortality (96h) and reproduction inhibition (49d) tests with *P. hawaiensis* were performed with whole sediment. The *Salmonella*/microsome was performed using a miniaturized protocol with strains YG1041, TA98, and TA100, in the absence and presence of metabolic activation. An exploratory non-target screening of organic fractions was conducted using UHPLC–Orbitrap HRMS. Low mortality was observed only for Praia Grande. In the reproduction test, organisms exposed to sediments from Praia Grande and Praia das Cigarras produced fewer neonates per surviving female than those exposed to Santos's sediment, and all sediment samples resulted in fewer neonates than the control. Sediments from Santos and Praia Grande were mutagenic for strain YG1041 with and without metabolic activation, while Praia das Cigarras showed mutagenicity only with metabolic activation, suggesting the presence of mutagenic substances related to aromatic amines and nitrocompounds in Praia Grande and Santos, and mainly aromatic amines in Praia das Cigarras. A total of 82 compounds were identified in at least one sediment extract: 32 in Praia das Cigarras, 14 in Santos, and 39 in Praia Grande. These compounds were classified into three categories: Pharmaceuticals and Personal Care Products (PPCPs), Industrial, and Others. Santos' sediment consisted mainly of sand and had the fewest detected compounds, possibly due to its lower organic matter content. These results indicate that higher organic matter content may contribute to greater retention and diversity of chemical compounds in the sediments. These results highlight not only the need for continuous monitoring but also the inadequacy of relying solely on primary treatment and dilution at sea, reinforcing the urgency for improved wastewater management strategies to reduce ecological risks in coastal environments.

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4.06.T-03 From source to solution: understanding antibiotic residues in an Indonesian reservoir

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The presence of antibiotic residues in the tropical reservoirs remain largely unknown. Lack of understanding is particularly clear in regions where the types, patterns, and sources of antibiotics are poorly documented. Understanding the occurrence and sources of antibiotics is essential for designing effective management strategies. This study assessed the occurrence, sources, future impacts, and mitigation strategies for antibiotics in the Cirata reservoir, Indonesia. In total 21 of 65 targeted residues were detected in water of the reservoir belonging to six different classes: sulfonamides, diaminopyrimidines, lincosamides, tetracyclines, fluoroquinolones, and amphenicols. Ecotoxicological risks ranged from medium to critical, particularly for the antibiotic residues: sulfamethoxazole, ciprofloxacin, norfloxacin, lincomycin, with ciprofloxacin posing the greatest threat to cyanobacteria. Moreover, the class of fluoroquinolones may also promote the selection of antibiotic-resistant bacteria. Based on their occurrence and risk, 12 antibiotics were prioritized. By developing and using the improved MARINA-Antibiotics model, we assessed the sources, future state, and effectiveness of management strategies. Direct animal manure discharge, primarily from sheep, cattle, and broiler farming, was identified as the dominant source of antibiotics. Under an economy-driven, strong climate change scenario (SSP5–RCP8.5), antibiotic concentrations were projected to remain ecologically risky. Strategies targeting livestock, crop, and aquaculture production, as well as human-derived sources, were evaluated for their potential to reduce antibiotic concentrations. Due to the link between antibiotic residues and nutrients, commonly used nutrient-based strategies were modeled to evaluate their potential in mitigating antibiotic residue levels. These

strategies could lower concentrations by up to 41% by 2050, and reductions of up to 70% become achievable when combined with antibiotic-specific measures. This study represents an important step toward a comprehensive understanding of antibiotic occurrence and sources in Indonesian reservoirs, offering critical insights to inform targeted interventions aimed at mitigating ecological risks and the long-term impacts of antibiotic residues on the reservoir. Moreover, this study can be helpful for developing management solutions for other reservoirs facing similar socio-environmental and climate-hydrological challenges.

4.06.T-04 Global Pilot Study: Monitoring Chemical Pollution under Low-Capacity Conditions

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Chemical pollution is a global challenge. Composition, sources, and impacts vary regionally, posing significant risks to water resources, human health, and ecosystems. Although data on chemical pollution are rapidly increasing in high-income regions, such as Europe and North America, information from low- and middle-income countries in Africa, Asia, and Central and South America remains scarce. Nevertheless, awareness of chemical pollution is growing worldwide, and dedicated scientists and practitioners are prepared to address these challenges within their countries.

In this context, a global pilot study emerged from the workshop “Solutions for the ONE WORLD: Monitoring Chemical Pollution under Low-Capacity Settings”, held in September 2025 at the Helmholtz Centre for Environmental Research (UFZ) in Leipzig, Germany, in partnership with the International Panel on Chemical Pollution (IPCP) and the World Water Quality Alliance (WWQA). The workshop brought together international experts in chemical monitoring, with a particular focus on the Global South, who established a global network to advance solutions to chemical pollution.

The overarching aim of the pilot study was to demonstrate the potential of chemical pollution monitoring under low-capacity conditions. Therefore, 300 water samples from urban, agricultural and pristine rivers (as a control) in 29 countries worldwide were investigated for approx. 500 target substances using liquid chromatography high-resolution mass spectrometry (LC-HRMS). The countries from which samples were collected include Argentina, Australia, Brazil, Canada, China, Costa Rica, Czech Republic, Ethiopia, France, Germany, Ghana, India, Italy, Japan, Kenya, Lebanon, Malaysia, Mexico, New Zealand, Nigeria, Pakistan, Republic of Korea, Serbia, South Africa, Spain, Sri Lanka, Sweden, Tanzania, and USA. Preliminary results show substantial variation in the occurrence and concentrations of organic micropollutants across the sampled sites.

In summary, the present study advances our understanding of micropollutant impacts by conducting the first coordinated global monitoring initiative in rivers with varying degrees of anthropogenic impact. Our vision is to contribute to the establishment of a growing global chemical monitoring network that enables more consistent data generation, fosters capacity-building, and supports the development of solution-oriented strategies to reduce chemical pollution worldwide.

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4.06.T-05 Beating data-hungriness: How molecule, company, catchment and organism dimensions facilitate derivation of principles and parameterisation of models in risk and life cycle assessment

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Setting: Over the last decades, anthropogenic pressures on the environment have dramatically intensified and diversified. Nowadays, we have to deal with 100,000+ pollutants, sites and species.

Relevance: While models are available, application to thousands of cases is severely limited by data gaps due to financial, ethical, disciplinary and other constraints. As an alternative, missing information can be obtained by linking parameters to well-known chemical, hydrological, socio-economic and biological characteristics, in particular size (e.g., molecular mass, company revenue, catchment area, body weight).

Objectives: Consequently, the overall aim of the present study is to obtain a cross-disciplinary suite of statistical and mechanistic relationships for environmentally relevant parameters (quantities) in assessment models as a function of size and other descriptors, underpinned by overarching scaling principles. We focus on pollutants, covering emission and fate in catchments and cities, accumulation in organisms and effects on communities.

Methods: Based on data from reviews, databases and articles, we derive empirical and theoretical relationships for rate, time, density and other quantities Y (parameters) as a power ($Y = a \cdot X^b$) function of company, catchment, organism and community size X . We cover e.g., emission of waste from companies, transport of water in river basins, circulation of blood in organisms and transfer of biomass in communities including the pollutants these processes generate, carry and degrade.

Results: First, we will report examples of statistical regressions obtained. For instance, emission of pollutants is related to engine, company and city size, comparing scaling of fossil fuel combustion to biomass respiration.

Flow of water through lakes, rivers, plants and animals is linked to their volume as well. Perfusion of organs by blood is an allometric function of body weight. Size distributions of micro- and macroplastic particles in water are compared those of organisms in aquatic systems (usually called size spectra). Most of the variability is explained by size but other global variables like temperature can contribute as well.

Conclusions: Identifying overarching principles based on shared system characteristics (e.g., size, dimension), the integrated suite of theoretical and statistical relationships obtained 1) profoundly advances our understanding and 2) crucially reduces data-hungriness of assessment tools.

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4.06.P Filling the Gap: Solutions for Monitoring, Modelling and Assessment of Chemical Pollution Under Data Scarcity and Low-Capacity Conditions

4.06.P-Th165 Low-Budget Field SPE Kit for Water Sampling

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Continuous releases of complex chemical mixtures have made the comprehensive monitoring and assessment of organic micropollutants in water resources more urgent than ever. Yet, the monitoring of organic micropollutants remains constrained by analytical, logistical, and financial limitations. Even in Europe, monitoring programs typically target only a limited number of regulated substances, leaving many chemicals unassessed. In the Global South and other regions, monitoring is even more scarce due to restricted access to advanced instrumentation (GC-MS, LC-MS/MS), high operational costs, and other resource-related barriers. Conventional approaches for analyzing organic micropollutants involve typically laboratory-based extraction after sampling, that can be prohibitively expensive, particularly for developing economies. This presentation highlights the development of a cost-effective sampling method that uses easily accessible devices and is suited for regions with limited resources. Because this method is considerably cheaper than existing alternatives, it can be implemented globally, allowing datasets to be compared across regions and providing valuable insight into the occurrence of chemical mixtures in the environment. The goal of this study was to develop and evaluate an inexpensive, reliable method for monitoring organic contaminants in water using a low-budget field sampling kit. During the method development, we (i) assessed chemical losses due to filtration using the kit, (ii) evaluated recoveries for the combined filtration and solid-phase extraction (SPE) step, and (iii) examined the stability of compounds stored on SPE cartridges under different temperature and storage durations

4.06.P-Th166 Monitoring Fuel-related Volatile Organic Compound exposure in Low-Capacity Contexts: Lessons from Petrol Filling Stations in South Africa

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Petrol, diesel, and their emissions release various hazardous chemical agents, including volatile organic compounds (VOCs) such as benzene, toluene, ethylbenzene, and xylene (BTEX) into the surrounding air. Petrol filling attendants and the public are exposed to these hazardous chemical agents (HCAs), many of which are linked to adverse health effects. Regulatory guidelines in the form of occupational exposure limits (OELs), such as those outlined in the South African Regulations for Hazardous Chemical Agents, 2021 (RCHA) for VOCs, aim to minimise risk for workers. Despite international research on fuel-related exposures, data from South African contexts remain limited. This study was conducted at a petrol filling station, where personal exposure to VOCs among petrol filling attendants was assessed using TraceAir® II passive badges placed in the breathing zone, following the NIOSH 1500/1501 method. Environmental monitoring was also performed by placing TraceAir® II passive badges on tripods positioned at 1.5 meters from the ground to measure VOC concentrations. The TraceAir® II badges were analysed for BTEX. Environmental conditions were also monitored during the sample collection period, and measured airflow, temperature, relative humidity and carbon monoxide. In addition, the cancer risk and hazard quotient (HQ) (non-cancer risk) were calculated according to USEPA guidelines. All measured BTEX 8h-TWA concentrations were below the regulatory limits set by the RCHA (2021). Personal exposure to BTEX was higher ($p < 0.05$) compared to area exposure monitoring results. All benzene samples under the low exposure scenario (3 days) exceeded the lifetime cancer risk threshold as set by the USEPA. Under the high exposure scenario (5 days), the majority of the samples also exceeded this threshold. Hazard quotient values for toluene and ethylbenzene were below 1, indicating minimal non-cancer risk. Xylene HQ values ranged from 0.695 to 3.517, suggesting potential non-cancer health concerns in some cases. Although BTEX levels were within regulatory limits, chronic low-level exposure and cancer risks from benzene, alongside xylene's non-cancer hazards, highlight the need for improved exposure control measures. These findings provide baseline data to support informed decision-making on worker safety.

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4.06.P-Th167 A Passive Sampling Study of Volatile Organic Compounds in Skopje: Seasonal Trends and Potential Emission Sources

Volatile organic compounds (VOCs) represent important urban air pollutants, contributing to photochemical smog formation and posing risks to human health. Skopje, the capital of Macedonia, is often ranked among Europe's most polluted urban centers due to consistently poor air quality. Using the passive sampling approach, this study demonstrates a practical method for VOC monitoring under data-scarce, resource-limited conditions. It provides the first comprehensive, year-long assessment of VOCs in Skopje's ambient air, conducted at five representative sites using Radiello passive samplers to evaluate monthly average concentrations, seasonal patterns, probable primary sources, and ozone formation potential (OFP). VOCs from different chemical classes were detected, including aliphatic and aromatic hydrocarbons, terpenes, and hydrocarbon derivatives. VOC levels were highest in winter, driven by traffic, domestic heating, and industrial activity, while summer concentrations were lower due to photochemical degradation and enhanced atmospheric dispersion. Analysis of BTEX, including toluene/benzene (T/B) ratios and correlation patterns, indicated that traffic was the dominant emission source, with additional contributions from industrial activities and waste management at specific locations. Principal component analysis further identified six source-related factors: mixed traffic, solvent and industrial emissions, biogenic sources, fuel evaporation, personal care products, and industrial/petroleum-related VOCs. Meteorological parameters also played a role in VOC levels and trends, with aromatic and aliphatic compounds showing negative correlations with temperature, UV radiation, and ozone, while fine particulate matter (PM_{2.5}) correlated positively with aromatic compounds and terpenes. OFP analysis indicated that highly reactive species, such as toluene, xylenes, C₆–C₃ substituted benzenes, and n-tetradecane, contributed disproportionately to ozone formation despite their lower ambient concentrations. Overall, this study provides essential baseline data on VOC levels, sources, and atmospheric behavior in Skopje, supporting evidence-based air quality management and targeted emission controls. The results underscore the need to address both mobile and stationary sources and are relevant for other urban areas in Macedonia and the Western Balkans. Future work in Skopje should expand monitoring to additional sites and assess health risks from long-term VOC exposure.

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4.06.P-Th168 Legacy and Emerging Pesticides in Fish from the Letaba and Inkomati Catchments in Southern Africa.

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The rapid population growth across Africa has increased the demand for food production, resulting in increased pesticide use. South Africa is among the top four pesticide importers in Sub-Saharan Africa, raising significant concerns regarding the contamination of freshwater ecosystems. Organochlorine pesticides (OCPs), historically used for agriculture and vector control, persist in the environment due to their high lipophilicity and long half-lives. Despite bans on many OCPs, residues remain in aquatic systems, potentially affecting non-target organisms such as fish. Similarly, neonicotinoids, introduced as safer alternatives, are now linked to adverse effects on aquatic biota, yet little is known about their bioaccumulation in wild fish species in Southern Africa. This study investigated 13 neonicotinoids and 19 OCPs in fish muscle tissue from the Inkomati and Letaba catchments. Only two OCPs, p,p'-DDE and p,p'-DDD, were detected, likely due to ongoing DDT use in malaria control. Concentrations were generally low and varied between sites, with higher values found in the Letaba catchment. Neonicotinoids were detected at low concentrations, with acetamiprid and imidacloprid being the most prevalent, although the concentrations were lower than those reported globally. Muscle tissue, representing long-term exposure, may underestimate total pesticide burdens due to lower lipid content. Findings suggest historical contamination and ongoing inputs from agricultural and disease control practices. Although concentrations were below levels of concern for vertebrates, this study highlights the need for continued monitoring and expanded tissue analysis to better understand bioaccumulation and ecological risks in Southern African freshwater systems.

Keywords: neonicotinoids, OCP, DDT, freshwater fish, bioaccumulation

4.06.P-Th169 From Monitoring to Human Health Assessment: Contaminant Burdens and Nutritional Benefits in Azorean Fish under the MoniPOL Programme

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Long-term contaminant monitoring programmes are essential for safeguarding public health in remote regions with high fisheries dependency. In the Azores, where fish consumption exceeds national and European averages and where volcanic and hydrothermal processes influence metal availability, robust baseline information on contaminant burdens in seafood remains scarce. At the same time, fish provides key nutritional benefits, reinforcing the need for integrated risk-benefit assessment.

The MoniPOL programme was established in 2022 to address this information gap by systematically evaluating both contaminant loads and nutritional quality in regionally important species. Species were selected according to landing prevalence, consumer preference and biological traits linked to contaminant uptake. Muscle and liver tissues were analysed for cadmium, mercury, lead and arsenic, alongside energy content, total fat, protein and moisture. This combined dataset enables the identification of sentinel species, supports long-term surveillance and provides evidence required for consumer guidance and policy development.

Marked interspecific variability was observed. Mercury concentrations exceeded EU limits in some deep-dwelling species, and cadmium levels in the Azorean limpet, a highly valued regional delicacy, surpassed the maximum regulatory threshold for molluscs by 137%, indicating a potential dietary risk for frequent consumers. Arsenic was consistently the most abundant element, especially in demersal species, reflecting natural geochemical conditions and indicating the need for continued assessment. Nutritional profiles also varied widely: some species exhibited favourable protein and energy content with comparatively low contaminant levels, whereas others combined lower nutritional value with elevated contaminant burdens, reducing their suitability from a risk-benefit perspective.

By generating the first integrated contaminant-nutrient baseline for Azorean fish, MoniPOL provides a practical and scalable framework for chemical pollution assessment in data-poor, low-capacity regions. The programme strengthens the scientific basis for transparent risk-benefit communication, supports fisheries and public health policy, and contributes directly to SETAC's objectives on improving human exposure assessment and enabling evidence-based regional management.

4.06.P-Th170 Sharing the experience from the Spanish Monitoring Programme on POPs in Support of the Stockholm Convention

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Effective environmental policies are mandatory in order to protect the environment from chemical pollutants. The Stockholm Convention is a legally binding treaty signed in 2001 with the aim to protect the environment and human health from Persistent Organic Pollutants (POPs). Long-term monitoring of POPs based on a coordinated network, with consistent methodology and analytical procedures, is highly valuable for understanding the spatial distribution of these contaminants and for establishing a baseline to evaluate the effectiveness of regulatory measures. This study aims to assess in Spain the distribution and chemical profiles of POPs in core environmental matrices (air and water) proposed by the Global Monitoring Plan in support of the SC. The assessment is based on a single coordinated sampling network: the Spanish Monitoring Programme on POPs. To date, passive air (PUF-PAS) monitoring data from 23 locations across Spain between 2008-2023 and surface water monitoring data from 8 sites in the Tagus and Douro River basins collected between 2013-2024 have been assessed.

Air monitoring sites represented a wide range of environmental settings, including remote, rural and urban areas, which allows for the evaluation of the anthropogenic pressure influence on POP levels and profiles. Higher concentrations were observed in urban and suburban areas compared to rural and remote sites, along with significant decreasing trends for most legacy compounds (PCDD/Fs, PCBs, PBDEs, DDTs, HCB), indicating the effectiveness of existing regulatory measures. Yet, slow decline rates for certain substances suggest ongoing environmental persistence. Surface water monitoring revealed marked spatial variability in PFAS concentrations, with higher levels at sites influenced by urban activity. While stable or decreasing trends were observed for some compounds, increasing patterns at specific locations point to potential ongoing inputs or

delayed responses to regulatory controls.

The assessment strategy of the SMP-POPs highlights the complementary value of air and water monitoring for characterizing pollutants' behavior, identifying hotspots and supporting integrated risk management strategies. This data set from Spain serves as a baseline for future assessments of temporal and spatial trends at global scale. Our findings reinforce the need for sustained and harmonized monitoring to track long-term trends and guide effective environmental policies under international objectives.

Disclaimer/Disclosure:

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4.07 From Complexity to Clarity: Next-Generation and AI-Driven Approaches for Smarter Assessment and Regulation of Chemical Mixtures

4.07.T-01 Strategies for Chemical Mixtures Assessment in Consumer Products: Integrating Realistic Exposure and Toxicity Mechanisms Using Korean Product Data as a Case Study

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Humans are routinely co-exposed to complex chemical mixtures through consumer products. However, traditional toxicity assessments typically focus on single chemicals, limiting our understanding of mixture toxicities. To make mixture studies applicable to risk management, both real-world exposure—including realistic combinations of chemicals and their mixing ratios—and the mechanistic basis of mixture toxicity need to be considered, rather than simply reporting mixture effects. This study aimed to develop an assessment strategy for chemical mixtures in consumer products that integrates both realistic exposure and toxicity mechanisms.

We first identified chemical combinations actually present in Korean consumer products and selected Triclosan and 1,2-Benzisothiazol-3(2H)-one (BIT), two widely used preservatives, as case studies. Cytotoxicity was evaluated using four human cell lines representing key target systems: HepG2 (liver), BEAS-2B (respiratory), SH-SY5Y (nervous), and Jurkat (immune). Predicted cytotoxicity of mixtures was estimated using concentration addition (CA) and independent action (IA) models. The observed effects of mixtures were then compared with predicted values through the model deviation ratio (MDR) approach.

For the realistic-exposure track, we combined cytotoxicity testing of 1:1 equimolar mixtures with exposure assessment based on the tested ratios to characterize product-related risks. Seven combinations exhibited synergism in one or two cell lines. Exposure assessment was then performed for consumer products containing these synergistic mixtures, including cleaners, laundry detergents, and deodorizing agents. For the mechanistic track, we tested EC20-based equitoxic mixtures together with mode-of-action analysis. Sixteen combinations exhibited synergism in one or two cell lines, and mixture effects were highly cell line-dependent. Synergism was more frequently observed in SH-SY5Y and Jurkat cells, likely reflecting differences in metabolic and antioxidant capacity and membrane-related toxicokinetics.

This study proposes an integrated framework for assessing mixtures in consumer products using two complementary tracks. By integrating the results from both tracks, this approach helps identify the mechanisms underlying mixture effects in products and assess whether these effects are likely to occur at realistic exposure levels, thereby informing the prioritization of mixtures for risk management.

Disclaimer/Disclosure:

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4.07.T-02 BPA-Equivalents: An Effective Tool to Assess Mixture Toxicity and Substitution Scenarios of BPA and its Alternatives

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Bisphenol A (BPA) is an industrial chemical and endocrine disruptor phased out in Europe and therefore replaced by often structurally very similar alternatives. Monitoring data revealed that humans and the environment are already exposed to mixtures of BPA substitutes. Due to their shared structure and estrogenic activity, concentration-additive effects are expected for such mixtures.

We designed seven multi-component mixtures of BPA and up to nine alternatives based on European surface-water concentrations and tested them in five in vitro assays that are relevant for BPA and its substitutes.

Measured mixture effects aligned well with predictions for concentration addition for cytotoxicity, estrogenicity, and mitochondrial toxicity. Therefore, BPA-equivalents (BPA-EQ) could be used as a simplifying metric for interpreting mixture effects and for substitution scenarios.

Adding the five most frequently detected bisphenols to BPA increased total concentrations nine-fold but raised BPA-EQs 24-fold for estrogenicity and 12-fold for cytotoxicity and mitochondrial toxicity. Substitution simulations showed that meaningful reductions in BPA-EQ were only achieved with near-inert alternatives.

As BPA alternatives accumulate in mixtures, it is important to assess their risks to the environment and to humans as a group. The BPA-EQ approach helps translate chemical exposure data to mixture toxicity and allows easier interpretation and communication of mixture effects to regulators. Bioanalytical equivalent concentrations referenced to phased-out chemicals also offer a simplified way to assess and evaluate the risks of different substitution scenarios.

Disclaimer/Disclosure:

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4.07.T-03 Assessment of the Ecotoxicity of 600 000 Mixtures Reveals only Negligible Differences Between Concentration Addition and Independent Action

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For predicting mixture toxicity, normally the Concentration Addition model (CA) is used. It assumes, for a mixture of substances, that they are acting in the same manner on the measured endpoint. It is unclear how high the deviation to reality can be where some compounds can affect the same endpoint through different modes of action. The model for dissimilarly acting substances is Independent Action (IA). Which model predicts higher toxicity, depends on the distribution function that is assumed, its slope and the mixture concentration. In practice the knowledge to apply IA for prediction is often missing though, as it requires full concentration-response curves to be available for every compound, which are rarely reported. Obtaining an IA prediction and the toxicity for the present substances for organisms from the aquatic trias of algae, invertebrates and fish, is limited by the scarce availability of dose-response parameters for single substances.

This study established a database of concentration-response relationships from standard assays with algae, daphnids and fish, to estimate the maximum and likely difference between the mixture toxicity predictions by CA and IA for real-world mixtures, compiled from large-scale monitoring databases. Toxicity prediction modelling was applied to derive chronic EC10 values for all mixture compounds for algae, daphnids and fish. For all mixture components, a random CRC-slope was sampled from the empiric distribution in a Monte-Carlo simulation. The simulation was built to obtain a realistic distribution of ratios between the EC10 values predicted by CA and IA.

The slope draws for all 610.326 mixtures show a similar distribution of IA/CA ratio across databases and organisms. In about 80% of cases CA is more conservative.

In reality most mixtures will most probably contain some chemicals acting similarly and some that are not. That means it is highly likely that the real prediction error is even smaller than what was found in the simulation. The selection between CA or IA, thus becomes largely irrelevant in view of other sources of uncertainty, like EC10 and concentration determination, and considering the underlying exposure dynamics. In that regard, our simulation results support the notion to use CA as the default first approach.

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4.07.T-04 Making sense of chemical monitoring data for environmental risk assessment: a stepwise scenario-based approach

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Modern analytical methods produce extensive datasets on the occurrence and co-occurrence of chemicals in the environment. Modern analytical methods generate extensive datasets on the occurrence and co-occurrence of environmental chemicals, yet this wealth of information remains underused for understanding chemical pollution. We propose a systematic, scenario-based evaluation of exposure data that clarifies whether specific environmental compartments are at risk, identifies suitable mitigation options, and provides strategic guidance for future monitoring and ecotoxicological research.

The approach starts with a rigorous problem formulation that distinguishes several key assessment questions. Risk quantification supports site comparison and ranking, with Concentration Addition serving as a robust first-tier model where data permit. Identification of risk drivers is essential for source allocation and targeted mitigation; component-based methods provide transparent rankings of each chemical's contribution. The framework further enables the identification of sensitive taxonomic groups, informing group-specific protection strategies. A comparison of analytical detection limits with hazard profiles highlights chemicals requiring improved analytical methods, while *in silico* tools such as validated QSARs help to flag chemicals lacking empirical ecotoxicity data. For components identified as potential risk drivers, experimental follow-up is warranted.

Current environmental risk assessments often hinge on a binary distinction between “risk” and “no risk,” still shaped by long-standing guidance documents. However, to support robust decision-making, it is crucial to delineate the uncertainty space between these categories. This is best achieved through contrasting scenario analyses that explore different assumptions and create edge-case boundaries for plausible outcomes. Wide divergences between scenarios indicate insufficient robustness or incomplete datasets, whereas minimal differences strengthen confidence in the conclusions.

Overall, the proposed framework maximises the value of existing monitoring data, supports mixture-aware and uncertainty-informed environmental risk assessments, and provides a flexible structure that can also be adapted to other complex exposure situations, including human health assessments.

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4.07.T-05 Combined Toxicity and Mechanistic Insights of Common Heavy Metal Mixtures

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Heavy metal contamination of soil, water, and air is widespread near manufacturing facilities, processing plants, landfills, and mining sites, leading to chronic exposure for surrounding communities. Although individuals are routinely exposed to mixtures of heavy metals, the combined toxic effects of these exposures are not well understood. In this study, we evaluated the toxicity of metal compound mixtures commonly detected at contaminated sites. Our results show that mixtures of nickel/arsenic/cadmium and beryllium/arsenic/cadmium exhibit greater toxicity than their individual components, indicating potentiated effects in combination. Mechanistic studies revealed that cadmium-containing mixtures significantly increased oxidative stress compared to single compounds, resulting in greater DNA damage. Furthermore, these mixtures induced higher levels of interleukin-8 secretion and promoted angiogenesis more than their respective components. These findings provide important insights into the enhanced toxicity of heavy metal mixtures prevalent at contaminated sites and underscore the need for further research on mixture-specific mechanisms to improve risk assessment and remediation strategies.

4.07.P From Complexity to Clarity: Next-Generation and AI-Driven Approaches for Smarter Assessment and Regulation of Chemical Mixtures

4.07.P-Mo283 Alternative Assays for Routine Toxicity Assessment

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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 Daphtoxkit 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 Daphtoxkit, 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 undoubtedly 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.

4.07.P-Mo284 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 use of bacterial bioassays to assess toxicity is well established attributed to the rapidity of ascertaining results and convenience of use.

Single species bacterial tests have been in common use across a wide range of industry and environmental applications.

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 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.

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 fresh water species from a variety of habitats.

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 by UK's Department for Energy Security and Net Zero (DESNZ) guidance integral to the regime of tests to be utilised.

4.07.P-Mo286 Smart Assessment of MASLD-Relevant Endocrine Disruptor Mixtures Using a Definitive Screening Design

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most widespread chronic liver disorders globally, and growing evidence suggests that exposure to endocrine disruptors (EDC) may contribute to its initiation and progression. Understanding the effects of complex mixtures is difficult because the number of possible combinations is large, their relative potencies vary widely, and potential interactions can obscure the identification of toxicity drivers. Efficient strategies are therefore needed to prioritise mixtures and to determine which individual EDCs most strongly influence biological responses relevant to MASLD. In this study, we developed a stepwise and resource-efficient workflow for mixture assessment based on in vitro screening combined with a Definitive Screening Design (DSD), a statistical design-of-experiments approach that allows detection of main effects, nonlinearities and selected interactions using relatively few tests. The zebrafish liver cell line (ZFL) was used as the biological model because of its suitability for evaluating metabolic and toxicological responses. For each chemical, concentration-response data were used to derive effect concentrations, which were then applied as standardised forcing levels in the mixture design. The DSD was constructed to explore mixture behaviour in only 17 experimental runs, and cytotoxicity following 48-hour exposure was quantified. Statistical model fitting was used to estimate the contribution of each chemical to the overall response. A demonstration study was conducted using a mixture containing perfluorooctanoic acid (PFOA), p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and allyl hexanoate (AHC). The workflow successfully distinguished the relative influence of each chemical and clearly identified perfluorooctanoic acid as the dominant driver of cytotoxicity, whereas the other two chemicals showed little to no effect within the tested range. No substantial nonlinear or interaction effects were detected, indicating that the mixture response was governed primarily by the linear contribution of PFOA. This proof-of-concept demonstrates that the integration of in vitro assays with a DSD provides a powerful and scalable strategy for early-stage mixture assessment. The approach is well suited for prioritising more complex mixtures relevant to MASLD and will be extended in future work using 3D spheroid cultures and mixtures identified through cohort studies.

4.07.P-Mo287 AI-Aided Meta-Analysis on Global Change Drivers-Triggered Airborne Microplastics-Related Human Pulmonary Toxicity

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Anthropogenic global change had substantially led to a surge in airborne microplastics (MPs). The potential human health implications, particularly the side effects on the lungs, are a growing concern. However, the magnitude and determinants of MPs-induced respiratory effects remain uncertain. While numerous studies have investigated this issue, a comprehensive understanding of the overall risk is lacking due to the heterogeneity of methodologies and outcomes. This study applied an artificial intelligence (AI)-assisted multilevel meta-analytic framework to systematically synthesize experimental findings from in vivo and in vitro studies published up to June 2025. Study quality was rated using a standardized risk-of-bias assessment tool. A total of 40 studies satisfying the eligibility criteria with low to moderate bias risk were included for the calculation of summary effect size. We found that the most significant increases of effective effect size were observed in inflammatory response following airborne MP exposure, with substantial heterogeneity across endpoints. Meta-regression analyses indicated that smaller MPs size, higher exposure concentrations, and longer exposure durations were key moderators of toxicity. Furthermore, random forest modeling identified MPs size as the primary predictive driver of pulmonary toxicity. Overall, the findings uncovered by this meta-analysis quantitatively demonstrate that airborne MP exposure elicits measurable pulmonary toxic responses across the biological models. Our findings provide crucial information for prioritizing and developing risk-based policies and protection mechanisms to safeguard public health on human pulmonary toxicity responses attributed to anthropogenic amplification of airborne MPs variability.

4.08 From Nanomaterials to Advanced Materials: Ensuring Safety in Innovation

4.08.T-01 Imogolites for agriculture & environmental plant protection; a challenge to assess and regulate

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Imogolite (Imo) is a natural clay mineral with tubular morphology composed of aluminum hydroxide (Al(OH)₃) and oxo-hydroxysilicate anion (SiO₃)OH. However, synthesised and modified versions have emerged of nanohybrids with organic and inorganic components used to fine tune inner and outer surfaces and properties. Hydroxylated Imo (Imo-OH) and methylated Imo (Imo-CH₃) with hydrophilic and hydrophobic inner surfaces, respectively, as well as hybrid materials functionalized with copper/copper oxide nanoparticles are such examples. These materials have been synthesized by CEA Paris-Saclay to explore their potential as plant protection products/fungicides in agricultural applications. Safety considerations early in the design process will ensure their safe use for protecting human health and the environment. However, introducing an added layer of complexity for regulators and assessors to determine potential risk is that these materials are advanced, multi-component, nano-enabled and also can act as nanocarriers. In aqueous solutions and test media strong Imo inter-nanotube interaction occurred leading to gel formation at concentration >0.5 mg/L. Unique direct interactions of this Imo gel with algae and *Daphnia* sp. resulted in organisms being trapped and such physical effects resulted in toxicity being recorded for the endpoint of immobilisation to *Daphnia* sp. (OECD TG 202) and growth inhibition in algae (OECD TG 201). For *Daphnia* sp., immobilization and physical entrapment occurred at concentrations >6.25 mg/L. Algal growth inhibition was also measured at concentrations of 3.1 mg/L. At the cellular level significant reductions in RTgill-W1 fish cell viability were only measured for the Imo-CH₃ materials. The interaction of the gel caused disruption of the plasma membrane, however EC₅₀ values were all above 100 mg/L indicating an overall low hazard potential for both the Imo-OH and Imo-CH₃ materials. Under these conditions the Imo presents a hazard through physical interaction effects rather than inherent chemical toxicity. Modifications of the material through Cu nanomaterial hybridization however introduced an additional hazard from this chemical component. Risk assessments, and indeed SSbD assessments, of these materials may require additional considerations to overcome challenges when hazard and fate testing (e.g. stability issues/gel formation/multi-component aspect) to ensure the generation of robust and relevant data to meet regulatory needs.

Disclaimer/Disclosure:

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4.08.T-02 Lights, Camera... Action? Challenges for *Daphnia* during Advanced Materials ecotoxicity testing

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Daphnia have been historically used for ecotoxicity assessments, including nanomaterials and microplastics, establishing an extensive understanding of toxicity and range of endpoints for comparison. As a result, a range of test guidelines have been established, including the OECD 202 acute immobilisation, and OECD 211 for reproduction (or chronic exposures). However, these standardised and widely used test guidelines, are likely to need adaptation to ensure they are accurately assessing advanced materials due to the complex nature of the materials in the exposure scenarios.

Commercial formulations of advanced materials have been used in the Macrame project, to explore risk assessments and toxicity endpoints of advanced materials in complex product matrices (AdMa@CM). Here, we highlight the use of some commercial dry lubricant sprays (for example for use on bicycle chains) and how these can impact *Daphnia* to feed into an environmental risk assessment for the product. Initial toxicity assessments were undertaken in flat bottom glass tubes, following the OECD 202 guideline of 5 neonates in 10mL of exposure solution with 4 replicates per exposure. For this material, we trialled adding the aliquot of toxicant to the test vessel before topping up with medium and adding the neonates, in addition to adding the toxicant last to determine if exposure order has a significant impact on the observed endpoints. In addition, we also trialled different ages of the *Daphnia* (4-6 days old) to determine if a larger individual is a) more visible and b) more likely to survive the exposure due to different developmental stages. Initial observations of the exposure to the panel of AdMa@CM showed nearly 100% mortality in all groups, excluding the control. When observed

under the microscope, the exposed Daphnia were still alive and attempting to move but couldn't due to the physical nature of the toxicants. This was observed in the majority of exposures, including at the highest concentrations, leading to adaptation to the test design to explore this further.

Highlights from this work include the systematic exploration of the challenges the AdMa@CM can present in acute aquatic exposures. Immobilisation of Daphnia exposed to mixtures of graphene based AdMa are readily immobilised due to the physical impendence of movement, as opposed to the typical toxicant response.

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4.08.T-03 Animal-Free Computational Pipeline for Deriving In Vivo Nanoparticle Safety Thresholds from In Vitro Data

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Ensuring the safe use of nanoparticles requires exposure limits that are biologically meaningful and human health relevant. Yet most existing safety thresholds are derived from animal studies, which are costly, time-consuming, ethically constrained, and available only for a subset of substances. Meanwhile, in vitro assays generate abundant toxicity data but lack a direct quantitative linkage to in vivo doses. As innovation accelerates, there is a critical need for scientifically justified methods that extrapolate in vitro responses of nanoparticles to organism exposure levels, enabling early-stage safety evaluations aligned with Safe and Sustainable by Design (SSbD) principles.

We present an integrated in vitro to in vivo extrapolation framework that translates in vitro nanoparticle toxicity into organism relevant exposure limits using harmonized dose–response modeling and physiologically based pharmacokinetic (PBPK) simulation. We compiled and harmonized nanoparticle toxicity datasets across mouse and human cell systems, yielding 72 human and 38 mouse dose–response experiments after rigorous pre- and post-dose response modeling screening. Using the dose response curve, we estimated BMD₁₀ potencies from each in vitro toxicity experiment.

To enable translation, we converted in vitro BMD₁₀ values ($\mu\text{g}\cdot\text{mL}^{-1}$) into organism level equivalent doses ($\text{mg}\cdot\text{kg}^{-1}$ body weight) using PBPK-derived delivery efficiencies. These translated values were benchmarked against 33 harmonized mouse in vivo studies, showing strong material-dependent quantitative alignment, especially for graphene oxide. Deviations for silica and TiO₂ reflected differences in particle size distributions and endpoint types between in vitro and in vivo experiments. Finally, applying the human PBPK model, we derived nanoparticle-specific human exposure limits. These predicted thresholds represent biologically plausible, human-relevant safety ranges directly grounded in in vitro data.

By linking harmonized in vitro assays with PBPK-driven in vitro to in vivo extrapolation, this work provides a transparent, reproducible, and animal-free pathway for deriving protective exposure limits for nanoparticles. The framework supports SSbD initiatives by enabling early-stage safety evaluation for nanoparticle innovations.

4.08.T-04 Ensuring Safe Advanced Materials – MACRAMÉ Recommendations for Test Guideline Development and Standardisation

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Innovation of advanced materials (AdMas) and application of AdMas-enabled products is rapidly increasing. Besides their exceptional properties and functionalities, AdMas raise scientific questions about their behaviour, safety and transformation along their life cycle. Advanced test methodologies for physico-chemical characterisation, safety testing and detection and quantification along the life cycle of AdMas and AdMas-enabled products are urgently needed to gain insights for scientists, innovators and also regulators. To keep pace with innovation, testing methodologies need to be developed or adapted in a timely manner, proven to be fit-for-purpose and advanced towards harmonised and standardised methods.

The EU-funded project MACRAMÉ gained valuable scientific and technical experiences during the development of methods for assessing inhalable carbon-based AdMas of various morphologies and commercial AdMas-enabled products. MACRAMÉ is fully aligned with the EU ambitions to secure the safety and sustainability of new chemicals, materials, products and processes striving for zero pollution and toxic-free environments.

Based on its experiences, MACRAMÉ has developed Informed Recommendations on the Needs for Test Guideline Developments and the Standardisation of Test Methods that go beyond the state-of-the-art developed methods. Diverse stakeholder groups were consulted to provide feedback in multiple ways to these informed recommendations. The recommendations are in line with the identified needs and gaps towards regulatory and policy requirements for AdMas, captured by MACRAMÉ in the Needs Assessment Report on Regulatory & Policy Frameworks. Needs and challenges mainly lie in addressing the whole life cycle of (products containing) AdMas. This requires combined imaging and analytical approaches to quantify transformed AdMas in situ in products and following release along the product life cycle. In addition, this requires life cycle thinking in material design and the development of sector-specific methodologies as well as tools for Safe-and-Sustainable-by-Design (SSbD), along with incentives to encourage their use.

The overarching goal of the MACRAMÉ Informed Recommendations is to clarify the specific need for test method development and standardisation of methods for use with AdMas. This should then enable regulatory testing of AdMas that is manageable by industry, acceptable to regulators and can ensure the trust of other stakeholders.

Disclaimer/Disclosure:

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4.08.P From Nanomaterials to Advanced Materials: Ensuring Safety in Innovation

4.08.P-We329 Exposure Alteration of Silver Nanoparticles by Key Member *Sphingomonas* in *Daphnia magna*

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Host-associated microbiomes often contain bacteria capable of interacting with metal ions in their local environment. This capability could play a fundamental role in shaping exposure to soluble nanomaterials as silver nanoparticle. This study investigated whether silver-resilient microbiome members influence the response of *Daphnia magna* to soluble silver nanoparticles (AgNPs), aiming to clarify how the microbiome shapes nanoparticle exposure and toxicity. Acute toxicity tests were performed using axenic, mono-associated, and naturally colonized *D. magna* neonates. Microbiome depletion increased neonate sensitivity to AgNPs, demonstrating a protective role of the microbiome. Two silver-resistant bacteria, *Sphingomonas yanoikuyae* and *Microbacterium* sp., were isolated from the *Daphnia* microbiome. *S. yanoikuyae* exhibited enhanced uptake of silver ions, whereas *Microbacterium* showed limited ion accumulation. Mono-association with *S. yanoikuyae* restored AgNP tolerance to levels comparable to naturally colonized neonates, while *Microbacterium* provided only transient protection, maintaining tolerance for 24 hours but not at 48 hours. Toxicity in naturally colonized and *S. yanoikuyae*-associated neonates was primarily driven by the particulate species, whereas axenic neonates showed a dominant contribution from ionic silver. Mono-association with *Microbacterium* slightly reduced ionic toxicity but did not fully restore the natural phenotype. Overall, our findings reveal that host-associated bacteria modulate AgNP toxicity through active silver uptake and alteration of ionic species. This active uptake hence shape the exposure to host and affects sensitivity of silver nanoparticles. This highlights the microbiome as a

crucial factor in nanoparticle ecotoxicology and emphasizes that host-microbe interactions influence the outcome of nanoparticle exposure.

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4.08.P-We330 Surface Coatings Regulate the Biodynamics and Trophic Transfer of Silver Nanoparticles: Insights from Au@Ag Core-Shell Nanoparticle

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Silver nanoparticles (AgNPs) is widely used in production and daily life, and its potential risks to the aquatic environment should be further monitored. The most directly related to the toxic effect is the biokinetic process within aquatic organisms. At the same time, when AgNPs enter the organism, they undergo a series of migration and transformation, especially the release of silver ions, which is considered the key mechanism of toxicity. Therefore, this study constructs a typical aquatic food chain (*Escherichia coli*-*Tetrahymena thermophila*) to explore the influence of surface coating on the biokinetic process under direct exposure in water phase and indirect exposure in food phase. In addition, by synthesizing Au@AgNPs with different surface coatings, this study can better distinguish the accumulation and transfer processes of particulate and released silver ions in organisms. The results show that surface coating regulates the surface charge of Au@AgNPs, which significantly affects its biokinetic and trophic transfer processes. In water phase exposure, the PEI-coated Au@AgNP accumulates the most in *T. thermophila* due to electrostatic interaction, and the particulate silver is more easily excreted than the released silver ions. In the food chain transfer, the PEI-coated Au@AgNP achieves the transfer from *E. coli* to *T. thermophila* in a relatively complete particulate form, showing the highest relative transfer efficiency of gold. However, the silver and gold transfer efficiency of PVP-Au@AgNP is the lowest, indicating a lower bioavailability, less transformation, and higher excretion efficiency. Moreover, the released silver ions show higher bioavailability than the particulate form in both exposure pathways. In conclusion, this study demonstrates that surface coating regulates the surface charge of AgNPs, affecting their biokinetic and transfer processes in both water phase exposure and food chain transfer. At the same time, when assessing the ecological risk of nanomaterials, the differences in the behavior of particle and ionic forms and the potential amplification effect through the food chain must be considered.

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4.08.P-We331 Toxicity Assessment of Silver Nanoparticles in Aquatic Organisms from Different Trophic Levels

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Silver nanoparticles (Ag-NPs) are widely used in medical, pharmaceutical and industrial applications, and pose a potential risk to aquatic ecosystems due to their possible release into the environment. Their presence in surface waters may negatively affect organisms from different trophic levels, including microalgae, rotifers and fish. This study evaluated the toxicity of Ag-NPs in three representative biological groups to characterize their differential sensitivity and the potential ecotoxicological effects associated with these nanoparticles. Toxicity tests with microalgae (*Raphidocelis subcapitata* and *Chlorella vulgaris*) and rotifers (*Brachionus calyciflorus* and *Lecane quadridentata*) were conducted in 24-well polystyrene plates using five concentrations prepared in species-specific media. Microalgae were incubated for five days and rotifers for one day. Embryo toxicity tests with zebrafish (*Danio rerio*) followed OECD Guideline 236, evaluating mortality, morphological alterations and hatching inhibition. Median lethal or inhibitory concentrations (LC50 or IC50) were calculated, and potential morphological alterations in rotifers were documented. Ag-NPs caused toxicity in all species tested. *D. rerio* embryos were the most sensitive (LC50 = 7.38 mg L⁻¹), followed by *C. vulgaris* (IC50 = 8.26 mg L⁻¹) and *R.*

subcapitata ($IC_{50} = 13.53 \text{ mg L}^{-1}$). Among rotifers, *B. calyciflorus* was more sensitive ($LC_{50} = 14.53 \text{ mg L}^{-1}$) than *L. quadridentata* ($LC_{50} = 18.87 \text{ mg L}^{-1}$). Reproductive rates decreased from 0.9 mg L^{-1} in *B. calyciflorus* and 2.35 mg L^{-1} in *L. quadridentata*. Microscopic observations revealed structural deformities in microalgae, alterations in the lorica and body constrictions in rotifers, and reduced hatching and multiple malformations in fish embryos. Overall, the results indicate differential toxicity across trophic levels and highlight the high sensitivity of early fish developmental stages. The observed morphological alterations, reproductive impairment and sublethal effects demonstrate that Ag-NPs pose a relevant ecotoxicological risk to aquatic organisms. These findings emphasize the need for responsible management of reactive nanoparticles to minimize their release into freshwater ecosystems.

4.08.P-We332 Evaluation of the acute toxicity of six nanoparticles used in industry and cosmetics in *Danio rerio* and *Chlorella vulgaris*

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Nanoparticles widely used in the medical, cosmetic, food, textile, and energy sectors include silver particles (AgNPs), copper oxide (CuONPs), zinc oxide (ZnONPs), titanium oxide and dioxide (TiONPs and TiO₂NPs), as well as hydroxyapatite nanoparticles (HAp NPs). In cosmetics, ZnONPs and TiO₂NPs are frequently used due to their ability to block ultraviolet radiation in sunscreens. Their increasing use raises the likelihood of release into the environment, making it necessary to assess their potential toxic effects on aquatic organisms. The objective of this study was to determine the acute toxicity and median lethal concentration of six types of nanoparticles in early stages of *Danio rerio* fish and *Chlorella vulgaris* microalgae. All nanoparticles were characterized using scanning electron microscopy, energy dispersive X-ray spectroscopy, hydrodynamic diameter, and zeta potential. Fertilized *D. rerio* eggs less than 2 h post-fertilization and axenic cultures of *C. vulgaris* were used, exposing them to five concentrations of each nanoparticle and a control. The bioassays were performed according to OECD guidelines (236 and 201) for acute toxicity in fish embryos and algae, and the median lethal and inhibitory concentrations (LC_{50} and IC_{50}) were determined at the end of the tests. In *C. vulgaris*, only hydroxyapatite nanoparticles did not induce population growth inhibition. AgNPs and ZnONPs showed the highest toxicity, with median inhibitory concentration (IC_{50}) values of 3.06 and 4.23 mg L^{-1} . In *D. rerio*, only AgNPs and TiONPs caused mortality, teratogenic effects, and hatching inhibition. CuONPs, TiO₂NPs, ZnONPs, and HAp NPs caused hatching inhibition at concentrations of 3.12, 6.25, 12.5, 25, and 50 mg L^{-1} , but showed low mortality (?15%) at the highest concentrations evaluated. These results highlight important differences in the sensitivity of aquatic organisms exposed to nanoparticles widely used in industry and cosmetics and provide relevant information for risk assessment and the development of regulations aimed at minimizing their environmental impact.

4.08.P-We333 Environmental Implications of Nano-Enabled Rapid Antigen Test Kits: A Cross-Species Ecotoxicological Assessment

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Nanogold (nAu) is the most researched advanced material in biomedicine due to its unique physicochemical properties, modifiability, and perceived biocompatibility. Colloidal gold rapid test kits (RATs), including those for pregnancy testing, ovulation monitoring, drug use screening, HIV diagnostics, and more recently, large scale screening during the Covid-19 pandemic, are one of the most common applications of nAu due to their sensitivity and ease of use. However, as the use of such test kits and other sources of advanced materials increase, so does the prevalence of these materials in the environment, which could potentially threaten aquatic and human health. Despite this, there exists a large knowledge gap regarding the bioaccumulation, potential immunotoxicity, and stress-related effects of leached nAu. This study aimed to assess the characterization, toxicity, biodistribution, and physiological impact of nAu leached RATs in *Daphnia pulex*, *Daphnia magna* and zebrafish (*Danio rerio*) embryos using a OECD guidelines. Dynamic light scattering, Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS) and inductively coupled plasma mass spectroscopy was used to characterise the nAu leached. Zebrafish embryos had several lethal endpoints at low dose exposures, while behaviour was significantly affected in all species assessed. The brand of RAT used within the same test type affected the toxicity level. The primary gold nanomaterial (nAu) particle size was found to be 2-16 nm,

surface functionalization of the RATs shifted after use, and the concentration of nAu exhibited almost double leaching potential from conjugated pad after buffer addition (pH 4.5) in used RATs compared to the unused ones. Notably, no significant difference was observed in the leaching of used and unused kits at alkaline pH. These findings strongly suggest that RATs can significantly contribute to nano-waste pollution but highlight how minor changes within their development and production can be harnessed to ensure the safe and sustainable design of advanced materials.

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4.08.P-We334 Hormetic Responses of *Raphidocelis subcapitata* to TiO₂ Nanoparticles: Role of Reactive Oxygen Species and Metabolic Reprogramming

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Ecological risk assessment (ERA) of ENPs typically focuses on toxicity, yet evidence shows that low concentrations can stimulate growth. Predicted environmental levels of engineered nanoparticles (ENPs) often fall within these sub-toxic ranges. Hormesis, stimulatory effects at low doses, could occur below conventional toxicity thresholds, but its prevalence and mechanisms in photosynthetic microorganisms remain largely unexplored. We investigated the biphasic dose-response of the green alga *Raphidocelis subcapitata*, a typical species used in ecotoxicity tests, exposed to TiO₂ nanoparticles (TiO₂-NPs) with concentration ranging from 0.5 to 100 mg L⁻¹ for 24, 48, and 72 h and assessed growth and reactive oxygen species (ROS) generation. Biphasic dose-response curves were observed at 48 and 72 h, but not at 24 h, with EC₅₀ values increasing from 16.2 ± 0.4 mg L⁻¹ at 24 h to 28.3 ± 1.16 mg L⁻¹ at 72 h, likely due to NP aggregation, sedimentation, and shifts in the cell-to-nanoparticle ratio over time. At 72 h, TiO₂-NPs induced a pronounced hormetic response, with maximal growth stimulation (MAX) at 13 mg L⁻¹, enhancing up to 60% of the population, a zero-effect point (ZEP) of 0.5 mg L⁻¹, and a no-observed-adverse-effect concentration (NOAEC) of 21 mg L⁻¹. ROS measurements indicated mild oxidative stress at low TiO₂-NP doses, which was mitigated by cellular antioxidant defenses at stimulatory concentrations. Targeted metabolomics revealed dose-dependent reprogramming of amino acids, nucleotides, fatty acids, carboxylic acids, and antioxidants. At ZEP, metabolites associated with amino acid metabolism, the TCA cycle, and antioxidant pathways accumulated, whereas at NOAEC, these metabolites were depleted. Notably, depletion of TCA intermediates, amino acids, and nucleotides began at MAX and intensified at higher TiO₂-NP concentrations. These findings support the hypothesis that mild oxidative stress drives hormesis growth stimulation, with ROS accumulation and metabolic adjustments acting as key mediators. Overall, this study highlights the ecological relevance of sub-toxic stimulatory effects in microalgae and provides mechanistic insights into how TiO₂-NPs modulate growth, oxidative stress, and metabolism. By providing a rigorous and comprehensive evaluation of the response to ENP exposure by phytoplankton, the base of the food chain, we aim to provide the basis for improved ERA and safer-by-design nano-enabled products.

4.08.P-We335 Ecotoxicity, Ingestion and Depuration of Nano-Enabled Fertilizers in Algae (*Raphidocelis subcapitata*) and Crustaceans (*Daphnia magna*)

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Controlled release nano-fertilizers (NF) are a promising alternative for improving nutrient use efficiency and soil quality. However, nanoforms remain a challenge due to their dynamic behavior in test systems. Consequently, careful characterization of the exposures is pivotal for correct interpretation of the test results. In this work, we assess the ecotoxicity and bioaccumulation potential of two nanocarriers (nanoclay bentonite & silicon dioxide) with two individual fertilizers (NH₄NO₃ & KCl) all functionalized with hydrophobic deep eutectic solvent (HDES). Suspensions of the nanoforms were prepared following OECD TG 318 and particle size and zeta-potential were characterized with Dynamic Light Scattering (DLS). Exposure concentrations were quantified using inductively coupled plasma mass spectrometry (ICP-MS). For ecotoxicity, each component was evaluated individually and in combination using the freshwater algae (*Raphidocelis subcapitata*) and the

crustacean (*Daphnia magna*) with OECD TG 201 and 202 respectively. Bioaccumulation potential was assessed in *D. magna* neonates using an uptake period of 24h, following depuration for 24h, and an additional 24h depuration period in the presence of a food source (*R. subcapitata*).

Stable suspensions of nanocarriers were prepared in MilliQ with z-average in range from 200-250 nm and a PDI of 0.4-0.6. However, the suspensions of the final formulations were heterogeneous with ranges from 600 to 2700 nm and PDI between 0.8-1. Additionally, visible sedimentation was observed after addition to media indicating an unstable exposure. Nanocarriers inhibited the growth of algae ($EC_{50} = 237$ mg/L and 107 mg/L for NCB and SiO₂, respectively) while no immobilization of *D. magna* was found for exposure concentrations up to 1000 mg/L. No effects were observed for HDES, fertilizers, and fertilizer-impregnated carriers. The final NFs showed growth rate inhibition of algae (EC_{50} values ranging from 33 to 94 mg/L) and immobilization of *D. magna* (EC_{50} values ranging from 27 to 68 mg/L). The mixture toxicity of the final formulations (toxic units) was calculated based on experimental and theoretical values for the individual substances. Assuming concentration addition, 64% of the response could not be explained. Further studies are necessary to investigate the discrepancy between the individual substances and the final formulations especially focusing on potential particle-specific effects.

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4.08.P-We336 Safety and sustainability of innovative materials – the communication and knowledge base MANTRA

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Innovative materials are needed to remediate environmental pollution, or secure green energy transition, to give just a few examples. Specifically, innovative membranes allow the removal of hazardous substances from waste streams, and catalysts enable the low energy production of green hydrogen. Novel materials and technologies are also needed to replace or reduce the use of rare or otherwise critical elements in industrial catalysts.

However, it is not enough to consider only the efficacy and efficiency of novel materials; their safety for humans and the environment, as well as their overall sustainability, must also be guaranteed.

The project MANTRA (Data on Innovative Materials for Sustainability and Transfer) supports research teams in developing promising materials and processes for membranes and catalysis. MANTRA supports activities in science communication, networking, public relations, the systematic selection and application of established sustainability indicators, LCA, advice on material safety, and the transfer of results to industry and practice. MANTRA also provides knowledge about the health effects and sustainability of new catalytic and membrane materials accessible to the public. This is achieved through a dedicated website (<https://materialneutral.info/en/>), brochures, information flyers, regular status meetings, workshops, and participation in conferences. These activities facilitate the exchange of information on material safety and sustainability among all relevant stakeholders, from material developers to end users.

The proposed contribution will present the MANTRA knowledge platform, introduce the scientists behind the project, and outline the goals and methods used to communicate the safety and sustainability of innovative materials.

Key messages:

1. Innovative, safe, and sustainable materials are essential for tackling today's societal challenges
2. The MANTRA project supports the development and adoption of such materials for membranes and catalysis
3. MANTRA addresses human and environmental safety of innovative materials, and the need to select appropriate, multidimensional sustainability indicators

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4.08.P-We337 Ecotoxicological effects of fibrous and platelet-shaped advanced materials on *Daphnia magna*

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The growing use of advanced materials (AdMas) raises concerns about their ecological impact, and the applicability of existing test guidelines to this specific group of materials. Fibrous and platelet-shaped materials represent advanced materials for which a reliable environmental hazard assessment based on the available test methods is challenging.

The project “FaPlaN” therefore aims to obtain information on the mechanisms of action (MoA) and thus a mechanistic understanding of potential toxic effects on aquatic organisms as green algae, cyanobacteria and daphnids exerted by fibrous and platelet-shaped advanced materials.

This contribution focusses on Boron nitride nanotubes (BNNTs), Boron nitride platelets (BNPs), silica carbide nanotubes, and MXenes regarding acute and sublethal toxicity in *Daphnia magna*. Alongside the incorporation of agitation to OECD test guideline 202, to enhance dispersion of materials during the test, feeding inhibition and oxidative stress biomarkers are assessed as additional endpoints. Further, the influence of particle morphology, developmental stage of the test organism, and exposure conditions on toxicity is evaluated. All test materials were fully characterized prior to testing, and material uptake was quantified to address size and shape-dependency, as well as dependency on daphnia life-stage. Miniaturized assays in 24 well plates were performed under static and dynamic conditions (shaking at 150 rpm), addressing limitations of the standard test guideline. Results showed that BNNTs induced the highest overall toxicity, while MXenes, although causing only minimal immobilization, led to significant feeding inhibition and redox imbalance, effects that would be missed by standard tests. Dynamic conditions generally increased toxicity by enhancing particle dispersion; however, this effect was not significant for all endpoints, particularly for BNNTs and BNPs. Age-dependent sensitivity was also evident, with 24 and 48 h neonates showing heightened more pronounced effects, compared to 4 d old neonates.

These results advocate for revising the current OECD TG 202 with regard to dynamic test conditions, to provide more continuous exposure of neonates to the test materials throughout the experiment. Further suggestions include to incorporate age-stratified testing, improved exposure scenarios, and multi-endpoint approaches.

Disclaimer/Disclosure:

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4.08.P-We338 Ecotoxicological Effects of Fibrous and Platelet-shaped Advanced Materials on Freshwater Algae and Cyanobacteria

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Advanced materials (AdMa) are a very heterogeneous group of materials and used in many applications. It is unclear, whether OECD test methods in the field of ecotoxicology (including those already adapted for testing nanomaterials) may be applicable for this class of materials. Due to their special morphology, fibrous and platelet-shaped materials represent advanced materials that might exhibit a specific mechanism of action (MoA) is unknown and a reliable assessment of an environmental hazard based on the available test methods is challenging.

Our project aims to check the feasibility of OECD test methods for AdMa und to receive information on their MoA for aquatic organisms as green algae, cyanobacteria and daphnids exerted by fibrous and platelet-shaped advanced materials.

Boron nitride nanotubes and platelets, a silica carbide (SiC) nanowire and a titanium carbide (TiC) MXene were tested to investigate potential MoA of the materials to algae. All materials were fully characterized. Preliminary tests were performed to consider shading effects at the fluorescence measurement to determine cell density.

Finally, the effect of the four materials on the growth of the green algae *Raphidocelis subcapitata* and the cyanobacteria *Synechococcus leopoliensis* was observed following the OECD Test Guideline (TG) 201 and the recommendations of the OECD Guidance Document (GD) 317. Next to effects on regular endpoints as growth

rate and yield, agglomeration behaviour between algae or cyanobacteria and the advanced materials was observed.

Only SiC nanowires and the TiC MXene had an impact on both green algae and cyanobacteria, indicating that green algae were more sensitive. Attachment of particles to algae appeared to be the most relevant MoA. Test methods (OECD TG 201, OECD GD 317) appeared to be feasible. However, at a high concentration of 100 mg/L a direct effect of the test material on the fluorescence measurement and therefore on the determination of the cell density could be observed. The measurement was impacted by around 20 to 40%. While there is a chlorophyll extraction method for the green algae *Desmodesmus subspicatus*, there is none for *R. subcapitata*. As a simple transfer of the method seems not feasible, there is the need for a broader discussion on the adaptation need of applying *R. subcapitata* in TG 201 to investigate the aquatic toxicity of advanced materials interfering with the measurement system.

Disclaimer/Disclosure:

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4.08.P-We339 An Integrated Approach to Evaluate the Safety of Innovative Materials for Water Treatment According to the SSbD Framework

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As nanomaterials (NMs) gain widespread use, quantitative frameworks integrating functionality, safety and sustainability considerations across their life cycle are needed. Since current sustainability methods overlook nano-specific hazards, strategies to incorporate toxicity data into Life Cycle Assessment (LCA) are required. Approaches based on Key Decision Factors (KDFs) and Key Performance Indicators (KPIs) allow linking design options to multidimensional performance, supporting early identification of safer and more sustainable configurations.

This study applies such a methodology, focusing on safety aspects, to novel NMs applicable to nano-enabled products (NEPs) such as water filtration membranes (WFM), and proposes a preliminary strategy to integrate the data into sustainability frameworks. Two case studies are presented: nanoforms and NEPs based on copper oxide nanoparticles (CuO NPs) and perovskite nanoparticles (Pv NPs), both applicable for antimicrobial/antifouling filtration systems. CuO-enabled WFM with or without a chemical binding (PVB) represent two design options, while four Pv NP types were analysed based on different synthesis parameters, defined according to a design of experiment (DoE).

Aquatic toxicity was assessed using zebrafish embryos (OECD 236) and larval behavior assays, while in vitro skin sensitization (OECD 442D) was also evaluated for CuO-WFM. The analyses generated primary data to support the development of nano-specific Effect Factors (EFs) for LCA. Interpreted through the KDF–KPI perspective, results indicate the PVB-containing WFM as the best design option. PVB enhanced antibacterial stability, improved NPs retention within the polymeric matrix and reduced in vitro toxicity while maintaining ecotoxicological safety. All tested Pv nanoforms showed promising application potential due to their favorable safety profile.

The findings show how structured KDF–KPI analyses enable comparison of design alternatives also according to a DoE, while the generated toxicity data build the quantitative basis needed for future nano-specific impact categories in LCA. Integrating a DoE approach into the KDF–KPI framework could further relate NM performance to synthesis-related KDFs, improving the derivation of EFs for LCA studies considering different synthesis routes.

Disclaimer/Disclosure:

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4.08.P-We340 Assessing the Host-Microbiome Interplane in Nano-Ecotoxicology: Moving Beyond an Endpoint

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Advances in sequencing and computational tools have greatly improved our ability to study the microbiome. Because the microbiome can either support or impair host health, understanding its role when exposed to emerging materials, such as nanomaterials, becomes increasingly important in grasping toxicity. Aforementioned, we conducted a systematic review and meta-analysis to identify common patterns in how nanomaterials affect host-microbiome systems across diverse models. This systematic review and meta-analysis of available 16S rRNA datasets revealed strong functional redundancy within microbiomes, meaning that microbial species can shift without corresponding changes in functional pathways. This underscores that function, not composition, may provide more meaningful insight into microbiome-mediated effects meaning that the microbiome cannot be another endpoint measured. Ample studies have tried to find causal relationships between host adverse outcome and the microbiome via gnotobiotic techniques. These studies hint at the direct influence of microbiome during exposure with nanomaterials yet it still remains a black-box on what mechanism are at stake. Furthermore, the often-used gnotobiotic organisms have their limitations as they are hard to maintain and cannot be generated for every model system. To further develop a mechanistic view of the microbiome in nano-ecotoxicology, we propose the use of microbial metabolite supplementation. The current study aims to deepen understanding of bioenergetic adverse outcome pathways during nanomaterial exposure and to examine how microbial signal molecules influence key events. This approach seeks not only to clarify gaps in the current bioenergetic AOP but also to assess whether supplementation is a viable tool for the microbial ecotoxicologist's toolkit.

Disclaimer/Disclosure:

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4.08.P-We341 A Regulatory Framework for Assessing the Variability & Safety of Nanoforms

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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 nanoform (NF) variability while ensuring comprehensive hazard evaluation and regulatory compliance, to streamline regulatory submission and evaluation.

Stage one of the project gathered existing frameworks and data and formed a draft decision-tree framework for identifying the acceptable variation of nanoform characterisers (e.g. size, surface area, surface functionalisation) that would lead to undifferentiable hazard, exposure and risk. Unlike sets of similar nanoforms, the developed framework aimed to only require nanoform characteriser information to group these nanoforms into a single nanoform.

Here we will present the decision-tree framework and its intended use. The framework was tested with case studies and external validation and results of both will be briefly presented here.

Further, we will take you on a "tour de nanoform" under the REACH regulation highlighting robust elements as well as some of the key areas for improvement. Covering in general data curation, data variability, the ECHA database, information type and information patterns as well as the perceived usefulness of some of the decision-trees.

It is clear from this research that substantial work is still required to form a robust framework for regulatory purpose, but also that the current framework builds a strong foundation by building upon previous work (e.g. NanoApp & GRACIOUS).

4.08.P-We342 Integration of the Eco-corona into Regulatory Nanomaterial Ecotoxicity and Fate Testing

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Nanomaterials acquire a so-called eco-corona on their surface when entering natural environments. This eco-corona, consisting of environmental biomolecules, can significantly impact the nanomaterial's fate and toxicity. The European chemicals legislations REACH requires nanomaterial manufacturers and importers to report on how nanomaterials transform in the environment. In the regulatory guidance, eco-corona formation is acknowledged as an important transformation process, yet no regulatory guidance is provided on how to test it. In this study, we identify and map methods for characterizing and testing effects of eco-corona, based on the findings from the MISTRA Environmental Nanosafety project, and provide recommendations for their integration into regulatory nanomaterial test guidance. We identified methods to study eco-corona formation outside organisms in solutions with natural organic matter or animal secretes (ex vivo) and bio-coronas formed inside organisms when passing through them (in vivo). We propose updating the regulatory guidance on nanomaterial ecotoxicity and fate testing with these methods and methodological considerations. Furthermore, we recommend including eco-corona considerations in the persistency, bioaccumulative and toxicity (PBT) assessment required by REACH. Jointly, this could support the implementation of information requirements on nanomaterial transformation, make nanomaterial ecotoxicity tests more environmentally relevant and lower the uncertainties in data extrapolation.

4.09 From Outliers to Insights: Transforming Environmental Risk Assessment through Effect Modelling

4.09.T-01 Movement for landscape models - Is now the time for a universal framework?

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Ecological risk assessments of plant protection products assess both exposure and effects. Much effort has gone into improving the modelling of both. However, the location of the individual relative to the area of application largely controls the exposure of the individual, and movement controls location. For many species movement means that exposure is not static, but highly heterogeneous over time. Specifically for non-target terrestrial organisms (NTTO) the selection of microhabitats through movement plays a crucial role for their exposure to plant protection products. While recommendations for good modelling practice have been issued for TKTD models, population models, and mechanistic effect models in general, the development of movement modules is often ad hoc. Here, we present the continuation of the developments of a framework for movement modelling since SETAC 2024, where a general framework for movement modelling was first presented.

Especially NTAs are a very diverse group and also exhibit diverse movement patterns. Moreover it has been found, that for NTAs the specific microhabitat which individuals use is crucial for their exposure to PPPs. A literature review showed that seven factors (Direction, Speed, Motivation, Energetics, Memory, Response to landscape features, Response to other organisms) cover most movement processes included in the models. However, not all models included all factors and model complexity ranged from no movement to correlated random walks and further to sophisticated models including responses to landscape elements and other organisms. Overall, models for terrestrial species were more complex than aquatic species and vertebrate models were more complex than invertebrates. This diversity to some extent reflects the ecology of the species modelled, and the role movement plays in exposure assessment.

During a workshop at ECEM2023, attended by ecological modellers with a broad range of expertise, a preliminary universal framework for movement modelling has been formulated and afterwards extended. This universal movement modelling framework will help the development and assessment of models. It is not the intention that all movement models must include all factors, but rather that it will help the modellers make informed decisions about what to include and what to exclude. When factors are excluded, justification should be given for why the factors are not considered to be important.

4.09.T-02 Margins-of-safety Predictions for the Risk of Measured Pesticide Mixtures from German Streams by Using GUTS Models

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Pesticide mixtures in the environment are attracting increasing attention for risk assessment. A monitoring study detected multiple active substances in small German streams with fluctuating pesticide levels that could influence effects on aquatic organisms. This study assesses the impact of measured pesticide mixtures on aquatic macroinvertebrates by using GUTS models. We applied the concept of concentration addition for

substances with similar modes of action (MoA) to account for cumulative effects, and the concept of independent action for those with different MoA. In total, we included 19 substances with three MoA covering a selection of insecticides and fungicides from the monitoring in our study. We considered neuromuscular and neuroactive substances as well as sterol biosynthesis inhibitors. Due to limited sampling in time, we developed three exposure scenarios with different assumptions about pesticide concentrations between samplings. The GUTS models are calibrated to one substance per MoA group and extrapolated across the entire MoA group using equivalence factors that represent differences in sensitivities. LP50 values quantified the risks of single substances and pesticide mixtures.

We observe an increase in risk of one to two orders of magnitude for substances within each MoA group relative to the reference substance. Generally, insecticides pose higher risks, especially neuroactive substances, than sterol biosynthesis inhibitors. The assumptions in the different exposure scenarios strongly influence risk predictions, and potential risk drivers across the included substances and monitoring sites were identified. Although we only cover a part of real-world substance mixtures by considering 19 of 75 measured active substances from the monitoring study, risk has already been identified. This highlights the importance of considering mixture effects in a future environmental risk assessment.

Disclaimer/Disclosure:

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4.09.T-03 Holistic Evaluation of Aquatic Data for Advancing Species Sensitivity Distribution Models

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Species Sensitivity Distributions (SSDs) are central to ecological risk assessment. However, the construction of SSDs can be compromised by fundamental assumptions about test designs and test species. We reveal potential problems and demonstrate how to resolve them through modelling.

Firstly, constructing SSDs often suffers from inconsistencies among toxicity endpoints (e.g., measured after differing exposure durations). The General Unified Threshold Model of Survival (GUTS) can harmonize exposure durations and account for sensitivity differences across exposure profiles. We analyzed datasets of three insecticides to calibrate GUTS-RED models across aquatic invertebrates and predicted EP50 values, exposure profiles causing 50% mortality, under different scenarios to construct scenario-specific SSDs. We show that exposure profiles influence species sensitivity rankings due to differences in uptake and elimination rates.

Secondly, SSDs assume that the sensitivity of a few tested species represents equal shares of sensitivities across a taxon. This assumption is questionable without prior understanding of the relationships among different species' sensitivities. To test the assumption, we applied multiGUTS, which extends GUTS by incorporating known relationships of GUTS parameters that link the survival responses of species sharing receptors and pathways. From a multiGUTS calibrated to eight test species, we simulated the survival response of the whole taxon (represented by 10,000 hypothetical species). The artificial SSD directly reflects the sensitivity of the taxon and reveals the representativeness of the eight test species. We compared the artificial SSD to the conventional SSD approach applied in risk assessment. In our example, most tested species consistently fell in the sensitive range, indicating they are representative for risk assessment.

Our study underscores the value of integrating empirical test results with suitability checks from modelling to enhance confidence in ecological risk assessments. Our modelling approaches support harmonizing toxicity tests across designs and species. This allows for a more comprehensive understanding of species sensitivity.

4.09.T-04 A-priori predictions of GUTS parameters? Estimating GUTS parameters with LCx values from public databases for QSAR development

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The application of GUTS models in environmental risk assessment is limited by the lack of raw survival data required for parameter calibration, blocking their use in mechanism-based QSAR development and routine effect assessment. This study proposes generating a-priori GUTS model parameter estimates using existing LCx, LDx, and NOEC values from public databases.

The methodology involves extracting effect levels (e.g., LC50, NOEC) and associated concentration/exposure duration data from entries in public databases such as the US EPA ECOTOX. The process begins by standardizing the data to a common unit (mg/kg) and creating exposure profiles for each chemical based on collected metadata. Associated effect levels are interpreted as time-anchored survival probabilities. All resulting data for the same chemical and life stage are then combined into a pseudo-experiment and fed as observations to the GUTS-SD model. Using Bayesian MCMC sampling, the posterior parameter distributions of the GUTS-SD model are estimated. The approach was validated using artificial survival data and derived LCx values for a theoretical chemical under controlled conditions.

The validation results demonstrate that GUTS parameters can be successfully estimated from reconstructed survival time-series derived from LCx values, provided there are sufficient endpoints and effect levels. As data availability diminishes, the uncertainty in parameter estimation increases.

In an application example, the study analyzed LCx and LDx values for adult and larval honeybees (*Apis mellifera*) from the US EPA ECOTOX database, estimating GUTS parameters for 154 datasets. Correlations between GUTS parameters (kd, m, b) and physico-chemical descriptors (water solubility, LogP) were explored, indicating potential value in incorporating GUTS parameters into QSAR development.

This study establishes the feasibility of inferring GUTS parameters from publicly available survival endpoints, offering a pathway to reduce animal testing in chemical effect assessments. The consistent compilation of GUTS parameter estimates for various species and chemicals can benefit both experimentalists and modelers. Further research should investigate the relationships between GUTS parameters and chemical descriptors to support mechanism-based QSAR models, potentially simplifying the development and application of toxicokinetic/toxicodynamic (TKTD) approaches in pesticide risk assessment.

4.09.T-05 Combining individual-based laboratory, semi-field, and field data with mechanistic models for enhanced certainty in environmental risk assessment

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Environmental risk assessment of pesticides can be significantly improved by integrating individual-based laboratory, semi-field, and field data through mechanistic effect models. This holistic approach leverages the strengths of each dataset type—combining the control and mechanistic insight of lab studies with the ecological realism of field data—to provide a more robust and comprehensive evaluation of risks to non-target organisms such as bees and earthworms. By calibrating models with laboratory data and validating them against semi-field and field observations, inconsistencies can be identified and addressed, increasing confidence in regulatory decisions. Case studies demonstrate that this integrative modeling framework enhances the ecological relevance and certainty of risk assessments, supporting next-generation regulatory frameworks and paving the way for the inclusion of non-animal data and more meaningful endpoints in environmental regulation. [2025 EA Co...taset new | Word]

4.09.P From Outliers to Insights: Transforming Environmental Risk Assessment through Effect Modelling

4.09.P-Mo288 Mechanistic Refinement of Tier 1 Ecotoxicity: A DEB-TKTD Framework for Robust Algal Toxicity Endpoints to Support EU Regulations.

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The current standard analysis of Tier 1 algal growth inhibition tests (e.g., OECD 201) relies on simplifying assumptions, such as instantaneous toxic impact and constant exposure concentration, which often lead to suboptimal data analysis and assessment. These analytical shortcomings result in high uncertainty, ECx estimations with unduly wide confidence intervals, and potential over-protectiveness in NOEC/LOEC determination, especially when results lack a "perfect" dose-response pattern. To meet the demand for more robust and reliable environmental risk assessment (ERA) data under EU REACH and future Pharmaceuticals in

the Environment regulations, we developed and validated a mechanistic Dynamic Energy Budget-Toxicokinetic/Toxicodynamic (DEB-TKTD) modeling framework. This framework successfully implements an algal DEB model with a reduced GUTS framework for damage accumulation, allowing for the explicit consideration of time-dependent effects and toxicokinetics (TK). By analyzing the totality of the time-series data from standard tests, including experiments with challenging or "troublesome" compounds, this approach delivers significantly tighter confidence intervals for No-Effect Concentrations (NECs) and ECx values. Furthermore, it provides a transparent and robust method to interpret results even when chemical degradation or speciation is substantial over the course of the test. This advancement delivers the necessary mechanistic, high-precision tool for deriving accurate Predicted No-Effect Concentrations (PNECs), critical for enhancing the efficiency and defensibility of regulatory ERA in the European Union.

4.09.P-Mo289 Evaluation of Potential EDC effects on Fathead Minnow Populations with DEB-IBM models

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We used a Dynamic Energy Budget-Individual-Based Model (DEB-IBM) to assess the population-level hazard of a plant protection product on Fathead Minnow (FHM). The study aimed to evaluate results from an Extended One Generation Reproduction Test (FEOGRT). The DEB-IBM approach tracks individual fish performance (growth, reproduction, survival) over time. The DEB model was calibrated with FEOGRT data. The experimental results indicated that, relatively to the control, FHM exposed to PPP X reproduced less (but not statistically significantly) and had an elevated gonadosomatic index, whereas growth appeared unaffected. These observations can be mechanistically explained by a single physiological mode of action (pMoA) in the model. Six-year population simulations confirmed that the median long-term population growth rate and the abundance of adult males were only marginally affected, if at all. Total and juvenile abundance showed slight reductions (8%), but the median exposed populations remained stable, staying within the 95–105% confidence interval of the control. The study concludes that the substance is unlikely to affect FHM populations under the tested conditions. This mechanistic modeling approach provides a streamlined, validated tool to extrapolate individual-level pMoAs to population-level risk without requiring a calibrated TKTD module, and is applicable to any fish species in the AmP collection.

4.09.P-Mo290 Mechanistic Modeling of the Antidepressant Bupropion's Impact on Daphnia magna populations: Meeting Future EU Pharmaceutical ERA Requirements

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The forthcoming EU pharmaceutical legislation and its strengthened Environmental Risk Assessment (ERA) requirements mandate robust, ecologically relevant data for human medicinal products, with the potential for refusal of marketing authorization based on inadequate assessment. This study provides a necessary mechanistic approach by using Dynamic Energy Budget (DEB) theory to model the effects of the antidepressant Bupropion on the keystone aquatic invertebrate, *Daphnia magna*. A Toxicokinetic-Toxicodynamic (TKTD) analysis identified the Physiological Mode of Action (PMoA) as a direct increase in costs for reproduction, which ultimately reduced individual survival and reproductive output. We employed a DEB-Individual Based Model (DEB-IBM) to extrapolate these effects to long-term population dynamics, using simulations with dynamic food, mimicking the feeding regime in OECD *Daphnia* reproduction studies. This mechanistic approach offers the high-tier, process-based evidence required to meet the stricter environmental sustainability criteria embedded in the new EU ERA framework.

4.09.P-Mo291 Beyond Single Stressors: Combined Effects of Polyethylene Terephthalate (PET) Microplastics and Food Limitation on Daphnia magna Population Dynamics and Implications for REACH Regulation

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Polyethylene terephthalate (PET) is an extensively used thermoplastic polymer, but a detailed understanding of its population-level adverse effects remains limited. This knowledge gap is critical, particularly as PET is being evaluated as a potential 'Polymer of Concern' under future EU REACH regulations, where multi-stressor data are essential for robust risk assessment. The goal of this study was to investigate the combined effects of PET microplastics and food availability on the population dynamics of a keystone aquatic organism, the water flea *Daphnia magna*.

We collected abundance data from *Daphnia magna* populations exposed to two concentrations of PET microplastics (125 and 250 mg/L, irregular particles <63 µm) and two food levels (high: 5 mg C/individual/d; low: 2.5 mg C/individual/d) for 60 days. A Dynamic Energy Budget Individual-Based Model (DEB-IBM) was validated using these experimental observations and subsequently employed to predict population responses to lower, more realistic doses of PET microplastics (1, 5, and 20 mg/L) under low food conditions.

Experimental results demonstrated that MP exposure caused a significant decline in population density and induced substantial changes to population dynamics. Model predictions confirmed that exposure to PET microplastics negatively impacts daphnid growth and reproduction, translating directly into decreases in population abundance. Crucially, both the experimental data and model simulations consistently suggest that food limitation significantly exacerbates the adverse effects of PET microplastics.

Our findings highlight the ecological complexity of plastic pollution and the necessity of integrating multiple environmental stressors into the ecological risk assessment of MPs. This integrated experimental and modeling approach provides essential population-level data, which is paramount for informing proactive risk mitigation strategies and the potential classification and restriction of PET as a Polymer of Concern under future REACH regulatory frameworks.

4.09.P-Mo292 Effect of Background Mortality Modelling Strategy on Environmental Risk Assessment of Chemicals Using GUTS Models

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Toxicokinetic–toxicodynamic (TKTD) models, such as the General Unified Threshold model of Survival (GUTS), are increasingly used in environmental risk assessment (ERA) for aquatic organisms. A key modeling decision concerns the treatment of background mortality (hb). Two strategies are commonly applied: (O1) fitting hb together with all other parameters, or (O2) estimating hb from control data and fixing it during subsequent fitting. While previous work (Plantade et al., 2023) showed little difference between these approaches for LC50 under constant exposure, their implications for ERA endpoints under realistic exposure scenarios remain unclear. We reanalyzed 38 survival datasets using a Python implementation of GUTS-RED (open source at <https://github.com/ibacon-GmbH-Modelling/pyGUTSred>) and computed LC50 and LP50 values. The LP50 represents the multiplication factor applied to time-variable exposure profiles (FOCUSsw scenarios) to cause 50% mortality at the end of the profile, as recommended by EFSA for higher-tier ERA. In total, 152 model fits (SD and IT variants) were combined with 10 FOCUS profiles, yielding 1520 LP50 estimates. Our results confirm that fitting hb to control data and then fixing it (O2) generally leads to more conservative endpoints: >60% of LC50 and LP50 values were lower with O2 than with O1. For the LP50, differences were sometimes amplified by the interaction between parameter values and exposure dynamics, with ratios exceeding 100 in extreme cases. These occurred in datasets with non-monotonic dose–response or poorly identifiable parameters, highlighting the need for biological plausibility checks and, in some cases, additional experimental data. Goodness-of-fit comparisons (AIC) showed no systematic advantage for either strategy. In conclusion, fitting hb to control data and then fixing it tends to produce more precautionary ERA outcomes, aligning with regulatory principles. However, parameter identifiability and exposure variability can strongly influence LP50 predictions, suggesting that guidance should consider both statistical fit and biological realism.

4.09.P-Mo293 SETAC working group on TKTD models for aquatic primary producers – status update

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The use of toxicokinetic-toxicodynamic (TKTD) models to assess the effects of time-variable exposure of organisms to plant protection products is now considered as an acceptable approach in most of the EFSA guidance in force. Since the publication of the Scientific Opinion of the EFSA PPR panel (2018) on the state of the art of TKTD models for regulatory risk assessment of pesticides for aquatic organisms, regulatory

submission of these models have increased, especially of the GUTS model for lethal effects. Some models are also available for assessing effects on the growth of primary producers (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. 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:

1) Development of a unified strategy for calibration, validation and extrapolation of TKTD models for primary producers

2) Create and test user-friendly, open-source software to support practical application

The working group has over 20 members and has been meeting regularly since autumn 2024. The poster will present the current state of work. The group has notably worked on the following tasks:

1) Review the checklist for primary producer models from the EFSA opinion for its practicability

2) Investigate the impact of the growth rate of the species on effects (conclusion: the high growth rate of *Lemna* is not a sufficient reason to question its representativeness for other, slower growing macrophytes under time-variable exposure)

3) Design of the mandatory sensitivity analysis for Tier 2C modelling studies (recommendation: perform combined uncertainty/sensitivity analysis with TKTD input parameter distributions reflecting their actual uncertainty from the calibration)

4) Model calibration: test different implementations and different calibration methods of the *Lemna* model (preliminary results: the three tested calibration methods yielded very similar parameter estimates and confidence intervals)

5) Calculation of statistically correct prediction intervals

4.09.P-Mo294 From individual to population modelling: A tiered ecological risk assessment of insecticide effects in *Chironomus riparius*.

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Mechanistic effect models (MEMs) are applied to refine risk at several tiers of the ecological risk assessment of chemicals. They should be designed as realistically as possible for a specific risk assessment question and, in the case of aquatic invertebrates, address population properties quantitatively as this is the relevant specific protection goal.

Density-dependent mechanisms are crucial for the regulation of stable, persistent animal populations, and their functional understanding is essential for the prediction and management of populations and must therefore be implemented in population models to describe their dynamics in the field. However, the greatest challenge in extrapolating to the field population is the lack of field data for the most relevant and sensitive environmental parameters (e.g., background mortality of larvae, loss of emerged adults, food availability).

We present a tiered modelling approach for the multivoltine, r-strategist taxon *Chironomus riparius* (Diptera), exposed to a hypothetical insecticide. The model framework integrates individual-based population models (IBMs) including a Dynamic Energy Budget (DEB) model, and toxicokinetic-toxicodynamic (TKTD) modules to connect exposure and effects and extrapolate laboratory data to real-world field scenarios.

To evaluate multi-peak exposures under various temperature profiles, we employed both the DEB-TKTD model with moving time window (MTW) for individual-level assessment and the more complex model for population-level field scenarios.

The population-level simulations offered additional insights beyond simple comparisons of maximum larval effects. It enables tracking of both larval density dynamics and cumulative adult emergence patterns over time. Furthermore, the model allows us to examine density-dependent population effects, which can potentially compensate for losses at high insecticide concentrations.

By incorporating temperature and size-dependent biological and toxicological processes, we analysed effects from exposure to the insecticide across multiple variables: exposure concentration, environmental temperature, and seasonal timing. This integrated modeling approach, combining individual and population-level responses under realistic conditions, delivers more thorough risk assessments for aquatic invertebrates - from worst-case individual life cycles to realistic field population scenarios - thereby reducing uncertainty in risk characterization.

4.09.P-Mo295 The SETAC MAPPED Workshop: Seeking How to Assess Population Relevance of Endocrine Disrupting Effects Using Models

Charles Hazlerigg¹, **Thomas G. Preuss²**, Andreas Focks³, Valery Forbes⁴, Udo Hommen⁵, Oliver Jakoby⁶,

Joachim Ulrich Kleinmann⁷, Laurent L.-M. Lagadic⁸, Kate Mintram⁹, Erik B Muller¹⁰, Melissa Reed¹¹, Pernille Thorbek¹², Charles Tyler¹³ and Lennart Weltje¹⁴, (1)Enviresearch Ltd., United Kingdom, (2)Bayer Research & Development Services LLC, Germany, (3)FB06/Institute of Mathematics, University of Osnabrueck, Germany, (4)Florida Atlantic University (FAU), (5)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (6)RIFCON GmbH, Germany, (7)BASF, Germany, (8)Bayer AG, Germany, (9)Brunel University, United Kingdom, (10)ibacon GmbH, Germany, (11)Health and Safety Executive (HSE) - Chemical Regulation Division, United Kingdom, (12)BASF, United Kingdom, (13)University of Exeter, United Kingdom, (14)Agricultural Solutions - Ecotoxicology, BASF, Germany

ECHA/EFSA (2018) states that models are one option for assessing the relevance of effects from Endocrine Disrupting Chemicals on vertebrate wildlife populations. An important step in such an assessment is defining what response in a population may be considered relevant. A creditable attempt at defining assessment criteria for use in risk assessment was presented in the EFSA (2023) Birds and Mammals guidance. This stated two criteria for concluding a low population-level risk using models: (i) the exposed population mean should not fall below the lower 95th percentile of the control and (ii) the lower 95th percentile of the exposed population should not be consistently below the lower 95th percentile of the control population (...on a limited number of points...). However, one encounters issues with these criteria when implemented in practice. For example, a population relevant effect can be identified using criterion 2, even when comparing two sets of control simulations without any chemical involvement at all (Tagliati et al. 2025).

As part of the SETAC MAPPED workshops (2024-2025), further options on how to assess population relevance in models have been explored for fish and will be presented. Some options explored revisions to the EFSA (2023) criterion 2, considering the use of alternative lower percentiles, as well as different frequencies and the consecutive nature of failing timepoints. These could be in isolation or in combination. Alternative approaches investigated population trends using census data over time, whilst others considered equivalence testing as opposed to more traditional hypothesis testing. Results from simulations with three fish population models (three-spined stickleback, brown trout and zebrafish) were used to inform the options explored and how they would address some of the ongoing challenges with defining appropriate criteria. The options presented may be used to inform risk assessors when making their decision on which approach may be taken in regulatory chemical assessment.

4.09.P-Mo296 Applying the Normal Operating Range Concept to Stochastic Models: A Robust Method for Detecting Population-Level Effects

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Mechanistic effect modelling is a valuable tool in higher tier Environmental Risk Assessments (ERA) of pesticides. Stochastic population models enable evaluation of potential population-level effects across diverse environmental scenarios and exposure patterns.

Currently, no explicit guideline exists for identifying pesticide effects from simulated population dynamics. The EFSA Bird & Mammal Guidance (2023) proposes two criteria for assessing low population-level risk: (i) the treatment mean abundance remains above the control's lower 95% confidence limit, and (ii) the treatment's lower 95% confidence limit is not consistently below the control's. Yet, no standardized method exists for applying these criteria to stochastic models.

We present a method specifically designed to detect the occurrence and duration of population-level effects in stochastic simulations, aiming to implicitly account for the above-mentioned EFSA criteria. Our approach constructs a model Normal Operating Range (mNOR) for the control mean abundance by bootstrapping the usual number of simulation replicates from a large number of control simulations. For each bootstrapping set, daily mean control abundances are calculated. The minimum and maximum values across these sets define the daily mNOR. Next, a daily mean treatment abundance is calculated based on one set of treatment replicates. The number of treatment replicates is kept comparable to that used for the bootstrap control sets to ensure consistency. Low population-level risk can be expected if daily mean treatment abundance remains within the mNOR for a relevant period.

We illustrate the proposed method using the stochastic, individual-based and spatially explicit simulation model eVole, which is frequently applied for higher tier ERA of small herbivorous mammals. We explore different scenarios and demonstrate how thresholds for acceptable effects can be integrated. We further derive daily confidence limits for both treatment and control, to explore how our approach relates to the proposed EFSA criteria.

Our approach supports recent efforts to improve ERA robustness by applying the NOR concept. Sensitivity to stochastic fluctuations is reduced by basing mNOR on mean values rather than extreme percentiles, while still

accounting for inherent variability in model outputs. This ensures robust detection of treatment effects and aligns with ideas posed in EFSA Bird & Mammal guidance.

4.09.P-Mo297 Acceptability of Mechanistic Effect Models in the Regulatory Environmental Risk Assessment of Pesticides: Considerations and Conditions from the SETAC MAD working group

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Mechanistic effect models (MEMs) dynamically simulate essential processes in ecological systems and how these processes are affected by chemicals (Grimm & Martin, 2013). The modeled systems can range from (sub)organisms, to populations, communities, or ecosystems. Recently, MEMs have been increasingly proposed for use in the regulatory environmental risk assessment (ERA) of pesticides. The work in the SETAC working group on 'Model Acceptability criteria and scenario Development (MAD)' has been focussed on the use of MEMs for the regulation of plant protection products in the European Union. So far, MEMs for regulatory purposes have been proposed mainly as refinement options, e.g., for assessing the effects of time-variable exposure on organisms or assessing effects on populations (EFSA PPR, 2013). However, MEMs can be useful exploratory tools to address various questions; this includes ranking the effectiveness of alternative risk mitigation measures or supporting the interpretation of ecotoxicological studies. In retrospective risk assessments, such models can be used to analyze mechanisms behind effects observed in (semi-) field studies and to support the establishment of appropriate assessment factors in ERA with an improved mechanistic understanding.

This contribution summarises considerations and background on the development and evaluation of mechanistic effect models, compiled by a multi-partite consortium with stakeholders from industry, contract research organizations (CRO), academic research institutions, and a number of European regulatory authorities. Such a multi-stakeholder group cannot define binding acceptability criteria for MEMs in the ERA of pesticides. However, it can provide input for further regulatory development that may be used to promote the development of such criteria and to identify critical research questions for the further assessment of MEMs and their use in the ERA of pesticides. The full extend of results is published in the MAD book (Focks et al., 2026). The proposed concept provides means to properly assess mechanistic effect models for their use in the environmental risk assessment of chemicals, here specifically of pesticides, because, if they are properly tested and applied, MEMs can improve ERA and make it safer and more relevant.

Disclaimer/Disclosure:

The views expressed are those of the authors and should not be interpreted as representing the official view of the Swedish Chemicals Agency.

4.09.P-Mo298 From Outliers to Solutions: Evaluating Insecticide Efficacy for Whitefly Control in Cotton

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The safe and efficient use of pesticides is critical for their registration, necessitating comprehensive field trials to evaluate the efficacy of formulated products across various cropping systems. In our presentation, we focus on the development of agronomic advice for managing whitefly infestations using the insecticide spidoxamat in cotton. The whitefly, *Bemisia tabaci*, poses a significant threat to agricultural productivity through direct crop damage and indirectly via the transmission of viral diseases. Although spidoxamat and other ketoenols generally demonstrate high efficacy against treated whitefly populations, some outliers observed in field trials suggest inconsistent performance influenced by environmental conditions and pest pressures.

To systematically address these outliers, we employed an individual-based modeling (IBM) approach integrated with a Dynamic Energy Budget (DEB) theory-based toxicokinetic-toxicodynamic (TK-TD) model. This integrated modeling framework allowed us to assess mortality rates of immature whitefly stages and the effects of spidoxamat exposure on the fecundity and fertility of adults as quantified in the greenhouse. We validated the

model using field trial data from diverse locations in India, Pakistan, and Brazil, enabling us to predict population dynamics and the efficacy of spidoxamat under varying field conditions. Our modeling efforts identified environmental scenarios that may lead to reduced performance of the insecticide and proposed optimal application strategies tailored to different pest pressures and temperature regimes. Notably, our results indicated that a second application of spidoxamat could significantly enhance population control, especially when administered between 7 and 14 days post-initial treatment. This timing is linked to the insecticide's efficacy half-life and the developmental duration of immature stages, reinforcing the necessity for precise application strategies in effective management of *B. tabaci* populations. Ultimately, our findings illustrate the critical importance of integrating empirical data with mechanistic modeling to turn outliers into actionable insights, thereby enhancing pest management strategies through effect modeling approaches.

Disclaimer/Disclosure:

All authors are employed by Bayer, which produces and sells agrochemicals including spidoxamat, the active ingredient of Plenexos®, investigated in this study.

4.09.P-Mo299 Quantifying Intraspecies Variability in Sensitivity to Toxic Effects to Improve Chemical Risk Assessment

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Chemical pollution poses a persistent threat to freshwater ecosystems, and assessing the effects of chemical substances on species and aquatic communities is central to chemical risk assessment. Species sensitivity distributions (SSDs) are commonly used to evaluate the ecological risks of chemical exposure, accounting for interspecies variation in sensitivity to toxic impacts. However, SSDs do not reflect the effects of chemical exposure on species abundance, considered an important endpoint in biological conservation. As a promising alternative, the change in mean species abundance (MSA) as a function of chemical exposure can be quantified by combining species-specific exposure–response relationships and population growth theory. Information on intraspecies variability in responses to toxicity tests is, however, not commonly reported. Here, we used regression models to predict intraspecies variability in responses to chemical exposure of ~3017 chemical-species combinations that have not been tested before. We first derived the ratio of the EC50 and EC10/EC20 Values of species-chemical combinations, based on existing toxicity databases, as a measure of intraspecies variability. After that, we explained differences in intraspecies variability via various regression models using species type (algae, invertebrates, and vertebrates), mode of action (e.g. (non)polar narcosis), and exposure duration (chronic vs acute) as predictor variables. As our model can be directly used to predict intraspecies variability, it provides great opportunities to integrate intraspecies variability into chemical risk assessment.

4.09.P-Mo300 Roles of Allometry, Trophic Guilds, and Phylogeny in Cross-species Sensitivity Extrapolation

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Most chemicals and species have never been tested, leaving both chemical potency and species sensitivities largely unknown. This knowledge gap poses a major challenge for modern ecological risk assessment's emerging focus on multiple species and multiple chemicals: How can we incorporate diverse species and their variation in sensitivity into community-level assessments when empirical data are sparse? Addressing this question is essential for linking species-level endpoints to community-level responses such as biodiversity and ecosystem functioning. To identify general principles governing cross-species variation in chemical sensitivity across the tree of life, we compiled an integrated dataset of 906 species (algae, invertebrates, vertebrates) and their sensitivities to 999 chemicals among six categories of chemicals, combined with information on species phylogeny, body mass, and trophic guild. We first used a machine-learning model to predict sensitivity for all species-chemical combinations. We then fitted Bayesian hierarchical models incorporating phylogenetic structure, allometry, and trophic guilds to evaluate their relative contributions in explaining species sensitivity. We found that species sensitivity to chemicals is strongly phylogenetically conserved, and models that include phylogenetic structure greatly outperform those that exclude it. All chemical categories exhibited a high phylogenetic signal, indicating that species with shared evolutionary history tend to respond similarly to chemical stress. In contrast, both global and taxonomic group-specific body size (allometric) effects were close to zero, suggesting that neither body mass nor trophic feeding guild systematically predicts sensitivity at this

broad scale. Our findings advance cross-species extrapolation and provide the bridge for linking species-level sensitivity to community-level responses, ultimately improving predictions of biodiversity loss and ecosystem functioning under chemical stress.

Disclaimer/Disclosure:

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4.09.P-Mo301 Population model for chemical and radiological hazards in marine biota: application to Atlantic cod in the Oslofjord

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Many radioactively contaminated sites are affected by both radiological and chemical pollutants, highlighting the need for consistent chemical and radiological impact assessment. Models to assess ionising radiation and chemical effects on wildlife are being developed. The challenge is to make these approaches practical for use in regulatory assessments.

In this study, carried-out as part of the activities of IAEA MEREIA Working Group 5 on mixed stressors in the Oslofjord, we evaluate a candidate approach to model dynamically in a practical scenario the effect of mixed chemical and radiological contamination in non-human biota. Building on work for IAEA and EC project RadoNorm, we extended our approach to a full toxicokinetic – toxicodynamic model by incorporating biokinetic transfer processes for waterborne radionuclides to fish and sediment, coupled to an ordinary differential equation (ODE)-driven logistic population model that can evaluate population-level impacts on mortality, morbidity and fecundity in a population of healthy and sick animals, with a common repair system for radioactive and chemical pollutants.

The model was set-up for cod (*Gadus morhua*) in the Oslofjord, considering Iodine-131 as the radionuclide and mercury (Hg) as the contaminant. The model inputs are the concentrations of the radionuclide (Bq/kg) and the chemical (mg/kg) in biota, along with biokinetic and ecotoxicological data. We also demonstrate how to include in the model multiple radionuclides and chemicals (e.g. heavy metals and organics), and how to consider not only additivity but antagonism/synergism in the mixture.

The focus of this study was on determining the key uncertainties and data gaps, and how the approach can be made useful for regulators in a multi-contaminant situation. Initial results indicate that increasing Hg concentrations beyond EU Environmental Quality Standards are the driving force affecting the population in the ecosystem of the Oslofjord. Current levels could potentially lead to tipping points with severe population decline. Parameter sensitivity analyses reveal significant data gaps (particularly in EC50 values), introducing uncertainty in predictions. Despite these limitations, findings suggest that toxic metals - especially Hg - pose an ecological risk, potentially amplified by radionuclide mixtures. The study calls for better ecotoxicological data and improved assessment models to support regulatory decisions and pollution control measures.

4.09.P-Mo302 toxdr: An R Package to Automate Dose-Response Curve Analysis

Jack Salole and Joanna Wilson, McMaster University, Canada

As test methods become more sophisticated, the complexity of toxicity test data becomes complex, and can consist of multiple endpoints across many technical and/or biological replicates. Currently, these values are used to determine effect measures (i.e. LC50, IC25) through dose-response modelling. While this process is well understood and can be completed with various programs, consistently applying it over many tests or experimental subsets is a tedious process. The ‘toxdr’ package is being developed to address this limitation of current programs. This package provides a set of modular functions that can intake raw data, perform quality

checks, apply user-specified transformations, fit and select the best fitting dose-response model, and determine the specified point estimates across multiple experimental subsets. Importantly, these modular functions are combined into a pipeline function that allows an automated process to analyze large datasets. This package is designed to support both quantal and quantitative endpoints, making it adaptable to diverse test measurements. This package is particularly well-suited for researchers working with large-scale toxicity studies that require efficient, consistent, and transparent data processing across large datasets. Publicly available versions of this package are currently being applied in research contexts to estimate effect measures. Here, we present an overview of the package and a case study using this package to rapidly analyze toxicity data from a dataset with 300 unique experimental subsets in under 5 minutes.

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4.10 Navigating and Harnessing Complexity in Ecological Risk Assessment of Chemicals

4.10.T-01 Navigating species diversity and data gaps: the role for trait-based approaches and modelling

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We present a trait-based framework combining systematic trait analyses for species selection and mechanistic modeling of field-relevant species in ecological risk assessments. Traditional focal species selection in pesticide risk assessments has been limited by its focus on presumed vulnerability traits and practical considerations, often missing potentially vulnerable species and lacking consistency. To address these limitations and data gaps, particularly for protected species where information is scarce, we propose a structured framework that supports the identification of representative species through trait-based analyses. We focus on two approaches: a supervised top-down method identifying traits associated with vulnerability, and an unsupervised bottom-up approach that groups species based on shared traits. The former assumes that protecting the most vulnerable species will safeguard less vulnerable ones and has been applied to pollinators, aquatic invertebrates, and wildlife species. The latter incorporates relevant traits through classification and categorization methods, such as discriminant analysis, non-metric dimensional scaling, and cluster analyses.

We demonstrate how these approaches can be applied to identify representative non-target arthropod species in European agricultural landscapes, where an estimated 3,000 species are present. The framework builds upon existing analyses of species traits and crop presence conducted by EFSA and industry groups.

A key extension of the approach is the incorporation of trait-based mechanistic modeling, allowing a single model to be applied across multiple species within the same trait group. These models can evaluate multiple scenarios and examine trait-stressor interactions, while maintaining consistent underlying model structure and processes. This approach enables standardized comparison across species without requiring separate model frameworks, as demonstrated in applications to solitary bees and cyprinid fish. The framework's adaptability makes it valuable for comparing risk assessments and management strategies across multiple species, especially for species with limited data. This flexibility extends across different ecosystems and geographical regions, informing and supporting ecological risk assessments of non-target arthropods - one of the most diverse taxonomic groups providing essential ecosystem services in agricultural landscapes.

4.10.T-02 APODEMUS, a Population Model for the Wood Mouse: implementation and model analysis

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The wood mouse, *Apodemus sylvaticus*, is a small mammal species that occurs across large parts of Europe. The species uses various habitats, has an omnivorous diet and can use pesticide treated agricultural fields for foraging. Thus, it is one of the focal species in risk assessments of plant protection products (PPPs) in the European Union. However, exposures and effects of PPPs on populations in the field are challenging to assess across different regions and landscapes. Population modeling has emerged as a valuable tool in higher-tier risk

assessments, complementing and extending data obtained from field studies. These models can simulate individual-level exposure and effects through modeled life-cycle processes, enabling predictions of population-level impacts. In this context, we introduce APODEMUS (A POpulation Dynamical spatially Explicit Model of the wood moUse). The model development was conducted using best practices, based on a comprehensive literature review and involved feedback from multiple stakeholders. The individual-based model represents the life cycle of the species using a Dynamic Energy Budget (DEB) model. The spatially explicit model considers the varying attractiveness of different habitats to the wood mouse. Density dependence can emerge from competition for space (represented by home ranges) by the mice. We applied the simulation model using an agricultural landscape whereby the habitat attractiveness was defined by expert opinion. We conducted a sensitivity analysis with the model, assessing the interaction of ecological parameters and the landscape scenario with model outputs including population abundance, structure and distribution across the landscape. These model outputs will ultimately be important to address scenarios including exposure to a pesticide in different agricultural landscapes and the comparison of model outputs to available wood mouse field study data conducted under different conditions. We present APODEMUS as a transparent and tested tool specifically designed for higher-tier mammal risk assessments. This model serves as a valuable complement to field studies by enabling the assessment of diverse environmental conditions, encompassing various landscape configurations and exposure scenarios.

Disclaimer/Disclosure:

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4.10.T-03 Chemical Co-Exposure and Risk Estimation for European Watersheds: A Combined Approach Using Models and Monitoring

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The EU Chemicals Strategy for Sustainability seeks to integrate environmental risks from “combined exposure” to unintentional chemical mixtures into regulatory assessments. ENCORE (ENvironmental CO-exposure and Risk Estimation, 2025–2027) is developing a probabilistic framework to assess mixture risks to aquatic ecosystems at the watershed scale across Europe. This approach synthesizes diverse information—chemical use, transport, degradation, fate, and hazard—while explicitly accounting for uncertainty in both exposure and hazard data. Building on the SOLUTIONS project’s large-scale exposure model, ENCORE applies object-oriented Bayesian Network (OBN) methodology to update prior probabilities from process-based simulations with monitoring evidence, improving prediction accuracy. Key data sources include pan-European datasets from the European Environment Agency: Waterbase Emissions (WISE-1) and Waterbase Water Quality (WISE-6). A pilot study using a subset of pesticides in Belgium demonstrates the implementation of this probabilistic approach, enabling exploration of alternative emission scenarios and mixture risk characterization methods. This proof-of-concept will inform scaling to larger European regions. Ultimately, ENCORE aims to identify chemicals that contribute most to mixture risk by incorporating spatial and seasonal exposure patterns across watersheds, supporting next-generation environmental risk assessment and management.

Disclaimer/Disclosure:

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4.10.T-04 ECOMIX: Understanding and Managing the Impacts of Mixtures of Chemicals and Co-Stressors on Mixtures of Species in English Rivers

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Rivers in England are in an unhealthy state. Only 16% of rivers meet the European Water Framework Directive for Good Ecological Status. While it is recognised that chemical pollution from wastewater emissions,

agriculture, highways and legacy mining activities is contributing to the problem, we still have a poor understanding of the real risks of chemicals and co-stressors in England's rivers. For example, current regulatory monitoring only focuses on a small number of chemicals in use, ecotoxicity testing focuses on only a small number of species that may not reflect sensitivity of the actual species that occur in English rivers, and chemicals assessment takes a single compound approach ignoring the fact that rivers are exposed to chemical mixtures and other co-stressors. The ECOMIX project is therefore developing a new catchment-based approach to chemical assessment and management that addresses these challenges. This approach: 1) assesses impacts of mixtures of chemicals and co-stressors on the structure and functioning of UK-relevant species assemblages at high spatial resolution; 2) considers the current situation and also looks to the future to account for the effects of global megatrends on chemical sources, fate processes, exposure and effects; and 3) allows us to target interventions where they are going to have maximum impact allowing us to benefit from the use of chemicals while protecting biodiversity. Focusing on the Yorkshire region in the North of England, the study, which has been running for over 3 years, is using a combination of continuous environmental monitoring, exposure modelling at high temporal and spatial resolutions, ecotoxicity testing on multiple algal and invertebrate species and hierarchical species sensitivity distribution and food web modelling to characterise the current and future risks of mixtures of over 40 high priority chemicals and co-stressors arising from different sources. This presentation will: provide an overview of the project approach; illustrate the results obtained to date on the occurrence, effects and risks of the case study chemicals in Yorkshire's rivers; and describe our plans for a catchment dashboard to support management efforts to improve the chemical quality of rivers in the region and to protect them into the future.

4.10.P Navigating and Harnessing Complexity in Ecological Risk Assessment of Chemicals

4.10.P-Tu380 Airborne particules and urban agriculture : optimization of health risk assessment

Sarah Boitelle, Ludivine Canivet and Annabelle Deram, Université de Lille, IMT Lille Douai, Université d'Artois, Laboratoire de Génie Civil et géo-Environnement (LGCgE), France

Urban agriculture presents health challenges linked to soil contamination and air pollution. While most studies have focused on soil pollutants, the contribution of particulate deposits from urban traffic to contamination of edible vegetables remains insufficiently assessed. This research aims to characterize the toxicity of these particles in relation to their physicochemical composition and better integrate their ingestion into human health risk assessments.

To achieve this goal, juvenile snails (*Helix aspersa*), well-established ecotoxicological models, were used due to their sensitivity to pollutants and ability to bioaccumulate contaminants. A total of 450 snails, sourced from a local farm, were randomly distributed into standardized experimental boxes after a three-week acclimation period in a vegetated environment (plantain, grasses, herbaceous plants).

Two exposure modalities were implemented:

- Dietary ingestion: incorporation of particles into a specific snail feed, allowing for chronic and homogeneous exposure;
- Ingestion via plant material: lettuce leaf fragments impregnated with particles, provided intermittently to ensure complete consumption.

Three types of particles were tested at five different concentrations, each with three replicates of four snails, resulting in a total exposure period of 28 days with biweekly feeding. Snail growth was monitored throughout the experiment. At the end of the exposure period, the snails were frozen and dissected for biochemical analyses.

The biochemical assays will focus on Major biological macromolecules (proteins, lipids, and carbohydrates), Oxidative stress markers (glutathione peroxidase (GPx), glutathione S-transferase (GST), and catalase (CAT)), Overall antioxidant potential (2,2-diphenyl-1-picrylhydrazyl (DPPH) method)

This experiment aims to highlight the differences in oxidative stress induced by the various types of dust particles. Ultimately, this may allow the different characteristics of the particles to be linked to the variations observed in the measured responses. The expected results will contribute to highlight a critical point of vigilance regarding the need to fully account for the ingestion of atmospheric particles deposited on vegetables —an exposure route that has so far been largely overlooked in environmental health studies—in health risk assessment protocols, in order to better reflect real exposure conditions in urban agriculture.

4.10.P-Tu381 PECsw refinement approaches in Environmental Risk Assessment for collyria medicinal products

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The Environmental Risk Assessment (ERA) evaluates the environmental impact of Human Medicinal Products (HMPs) based on their release into the environment via wastewater by excretion of drugs following their therapeutic use by patients. ERA is mandatory for all new Marketing Authorization (MA) applications for medicinal products. The guideline describes a step-by-step process based on the analysis of environmental exposure to the medicinal products, considering the worst-case dose regimen scenario. Phase I of the ERA includes the calculation of the Predicted Environmental Concentration in Surface Water (PECSW) of the medicinal products, taking into account the maximum daily dose (MDD), standard values of wastewater per inhabitant per day and of portion of treated population. If the calculated PECSW exceeds 0.01 µg/L, Phase II is necessary. 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 target disease/s and area, and on the worst-case treatment regimen specific for each disease addressed by the product. In the case of a collyrium, not all the product enters the body, but most of it drains out of the eye, onto the patient's cheeks during the administration. Therefore, the quantity of the drug that is actually absorbed by the patient during the treatment does not correspond to the MDD, but it is lower. Consequently, for the purposes of the ERA it would be relevant to consider some additional factors, such as the volume of a drop, the conjunctival sac capacity (often exceeding the average drop volume), the frequency of administration of the drops and the actual absorption rate of the eye, which is limited by the fact that only a small fraction of each drop is retained during ocular administration. These types of risk assessments need to be adapted based on approaches that, although consistent with the scopes of the ERA, are not addressed in the current reference guideline. In conclusion, it would be desirable for the competent authorities evaluating ERA submissions to remain open to accepting well-justified alternative approaches.

4.10.P-Tu383 Regional Variability of Pesticide Residues in Cottonseed and Fiber Across Türkiye's Major Agro-Ecological Zones

Volkan Mehmet ÇINAR¹ and Cafer Turgut², (1)Anadolu University, Türkiye, (2)Istanbul University, Türkiye

This study examines pesticide residues in cottonseed and fiber collected during the 2024 and 2025 seasons from Türkiye's major cotton-growing regions, highlighting geographical differences across areas. The sampling sites represent various ecological zones: the Aegean Region (Aydın and İzmir), the Mediterranean Region (Adana and Hatay), and the Southeastern Anatolia Region (Van and Diyarbakır). A total of 31 pesticides were detected in 2024, with 12 residues in cottonseed and 19 in fiber, indicating that fiber contains a broader range of pesticides. Acetamiprid, Chlorantraniliprole, Chloromequat, and Diuron were consistently found across all three regions, establishing the common residue baseline. However, residue diversity varied significantly by region. The Aegean provinces, especially Aydın, had the highest variety of pesticides, reflecting intensive farming systems and a long growing season that encourages repeated chemical use. The Mediterranean provinces exhibited a moderate but varied profile, with Adana showing a broader presence of pesticides in fiber, likely influenced by its warm climate and multi-cycle pest pressure. In contrast, the Southeastern Anatolia provinces—particularly Diyarbakır—had fewer seed detections, while Van showed notable fiber diversity, suggesting region-specific agronomic practices. Insecticides led in all three regions, followed by growth regulators, herbicides, and acaricides. Compounds like cypermethrin, lambda-cyhalothrin, malathion, and pyriproxyfen appeared more often in fiber, indicating different deposition patterns under varying climate conditions. Overall, the findings indicate that pesticide contamination in cotton is influenced not only by crop management but also by the ecological characteristics of Türkiye's different regions. These spatial variations highlight the importance of region-specific monitoring methods and localized pesticide-reduction strategies to improve environmental safety and protect consumers.

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4.10.P-Tu384 (Re)Establish the use of the Geometric Mean as Refinement Option for Chronic Aquatic Risk Assessments

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In the European Union, the risks of pesticides to aquatic organisms are assessed using a tiered system, whereby data on the acute and chronic effects of a substance on standard species (i.e. algae, macrophyte, aquatic invertebrate and fish) forms the basis of risk assessment (RA). The use of a geometric mean (geomean) was outlined as an acceptable refinement option for both acute and chronic RA in the EFSA Aquatic Guidance Document (2013). In recent years, this refinement option has been restricted especially for chronic tests (currently not accepted for chronic fish and aquatic invertebrates, Recurring Issues EFSA, 2018). Furthermore, its use as a refinement option for primary producers has also now been questioned by regulators and consequently could result in critical issues for the aquatic risk assessment. The main concerns over the use of the geomean approach from regulatory authorities are 1) the lack of comparability between tests regarding duration, endpoint and life stage; and 2) the missing verification of the protectiveness of the geomeans approach using results of the surrogate reference tiers. Conversely, a robust geomean approach could be more prominent in the future as there are also challenges on the use of Species Sensitivity Distributions (SSD). These challenges can lead to defaulting to Tier I risk characterisation due to the absence of alternatives for risk assessment.

To address the concerns from regulatory authorities chronic geomeans are calculated from existing regulatory data (across the 4 species groups). The data is used to assess comparability of different type of endpoints (e.g. length and reproduction endpoints in chronic aquatic invertebrate tests) as well as protectiveness in comparison to higher tiers as reference data (e.g. SSDs or mesocosm studies). To ensure the robustness of the geomean, reliability of endpoints used for the calculations will be investigated and any exclusion criteria determined and documented.

The outcomes of this analysis will inform the use of chronic geomeans and support transparent, protective aquatic risk assessments in regulatory practice.

4.10.P-Tu385 The challenge of deriving population model parameters from multiple data sets: the example of mortality rates in the wood mouse

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As indicated in multiple EFSA guidance documents, mechanistic effect models can be applied to provide scenario-specific temporal predictions of pesticide effects. In population models, reliable and adequate predictions require the representation of physiological and ecological processes of the species. However, even for well-researched species, knowledge is not complete, leading to simplifications of the model. For instance, models might not consider all covariables that likely influence a process in nature and are reflected in empirical measurements. Nevertheless, a reliable parametrisation can be achieved if data are compiled from available ecological studies which increases statistical power as well as coverage of the environmental context (concerning, e.g., different populations, climate, landscape, diversity or anthropogenic influence). However, empirical studies often employ different methods, describe different time scales, record different covariates, present data from different times and in different formats, and apply different statistical analyses. Thus, field data available must be harmonised, summarised and interpreted in the model context before they can be used for model parametrisation.

Here, we present an example for model parameterisation using the wood mouse (*Apodemus sylvaticus*). The population model APODEMUS developed for the species summarises the daily mortality rate of individual mice into a single parameter ('daily natural mortality'). This seemingly simple parameter is used in many population models. However, mortalities are reported in different ways in published studies. We demonstrate how the parameter can be consolidated from multiple sources. More specifically, we focus on field methodological differences (e.g. how is mortality/survival determined in the field?), differences in statistical evaluation (e.g. capture-mark-recapture models vs. minimum number alive approaches), and differences in the definition of important co-variables such as age or reproductive status that may impact mortality and other parameters. Using a single parameter from the wood mouse population model, we demonstrate the intricate process of model parameterisation that integrates data from multiple published sources. This example serves to illustrate a systematic approach for parameterising ecological models, highlighting both the challenges and methodological solutions in this complex process.

Disclaimer/Disclosure:

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4.10.P-Tu386 Predicting Wood Mouse Densities in European Agroecosystems: Insights from an Explainable Machine Learning Study

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Normal Operating Ranges (NOR) describe the natural variability of system properties such as population density or abundance and are increasingly recognized in ecological risk assessment as critical for interpreting population-level effects and improving model design. Recently, mechanistic effect models have been used to determine NORs, but it is not yet clear under which conditions they are suitable for this purpose. A complementary approach is to derive NORs based on available data to better understand this variability in the field. Here we present the results of an analysis focusing on a species important for mammal risk assessment – the wood mouse, *Apodemus sylvaticus*.

We compiled an extensive dataset on wood mouse population densities from peer-reviewed literature and industry field studies, resulting in 806 samples from 54 crop fields across the species' western European range. Data encompassed both agricultural and semi-natural habitats and included 84 explanatory variables describing crop characteristics, habitat structure, climatic factors, and experimental design parameters. Machine learning models predicting wood mouse densities achieved high predictive power (test $R^2 = 0.804$; restricted to predictions within the applicability domain: test $R^2 = 0.834$) and, using explainable machine learning techniques, key ecological, methodological, and agricultural drivers of wood mouse population estimates were identified.

Population density ranges were estimated as NOR surrogates in two ways: 1) via prediction intervals for context-specific model predictions (e.g., density range in a specific crop field on a given day of the year), accounting for both model uncertainty and inherent data variability, and 2) via aggregating predictions *inter alia* spatio-temporally (e.g., density range in cereal in southern Germany during summer). Both methods highlight substantial natural variability in wood mouse densities.

Our study delivers the first comprehensive synthesis of wood mouse population data in agroecosystems and contributes – also via the identified data gaps and methodological challenges – to developing more robust, evidence-based approaches for assessing and managing pesticide risks to small mammals.

4.10.P-Tu387 Optimal Complexity in Ecological Modelling: Application of a Novel Framework to Evaluate Biological Realism of Earthworm and Wild Pollinator Population Models

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Ecological models can support land management decisions and ecological risk assessments that need to account for invertebrate population responses at the field to landscape level. Models that incorporate greater biological and environmental detail (i.e. individual-level physiology and behaviour, fine-resolution environmental heterogeneity) have a greater capacity to represent emergent population dynamics, especially under novel conditions. However, more complex models risk high uncertainty, loss of interpretability and excessive computational demand, particularly at larger spatial extents. This trade-off between realism and tractability poses a long-standing challenge in ecological modelling and motivates the goal of determining optimal model complexity. In practice, models tend to be developed on an ad hoc basis, with structures optimised for specific species, scenarios and spatial scales, hindering generalisable insights needed to advance predictive ecological models. We developed and applied a novel approach to quantify the biological realism of models for two non-target invertebrate groups. Mechanistic and process-based population models for earthworms ($n = 23$) and wild pollinators ($n = 24$) were identified through a structured review. We find that earthworm models are predominantly non-spatial or micro-scale (<10 m extent) and often incorporate physiological mechanisms, whereas pollinator models are typically implemented across landscape to regional extents and focus on individual-level or aggregated movement behaviour. Our results highlight structural trade-offs that reflect differing priorities in model development for these taxa. Greater integration of physiological and behavioural mechanisms across broader spatial extents would, however, enable models to better represent phenotypic plasticity in spatiotemporally heterogeneous environments, supporting more informed predictions of population responses in agricultural landscapes. Systematic model testing is a crucial next step to quantify relationships between model structure, system behaviour, computational demand and predictive performance across spatial scales, providing evidence for where higher biological realism is important and where efficiencies can be gained.

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4.10.P-Tu388 Risky Buzz-ness: Navigating ECHA's Bee Guidance for Biocidal Product Risk Assessments *Helen Sneath, Don Bealing and Samantha Walker, Sagentia Regulatory, United Kingdom*

ECHAs Guidance on the assessment of risks to bees from the use of biocides entered into force for both active substance and product applications on 1st February 2026. Aligned with the EFSA guidance for plant protection products, the new ECHA guidance represents a significant change in approach for biocidal risk assessments. We document our first experience of applying the guidance to biocidal products, highlighting practical considerations, data requirements, and interpretation issues. Lessons learned provide valuable perspectives for stakeholders preparing to navigate bee risk assessments for biocidal product authorisations.

4.10.P-Tu389 Evaluating Changes in PT18 (Insecticide) Emission Calculations: Comparison of the 2008 and 2024 Emission Scenario Documents *Mathew Jackson, Sagentia Regulatory, United Kingdom*

Environmental risk assessment (ERA) of insecticides under the Biocidal Products Regulation (BPR) requires calculation of local emissions into the environment, allowing subsequent Predicted Environmental Concentration (PEC) derivation and risk characterisation. Such calculations are performed in accordance with assumptions and equations presented within the Product-Type 18 (PT18) (insecticides) Emission Scenario Document (ESD). Since the publication of the original PT18 ESD in 2008, there have been substantial developments in data availability and modelling approaches. The updated (December 2024) PT18 ESD introduces significant methodological revisions aimed at improving realism, transparency and regulatory harmonisation of insecticide ERA across Europe. Consequently, differences in the equations and resulting outputs of emission calculations exist between the original and updated ESD. This study investigated such differences in emission calculations between the 2008 and 2024 versions of the PT18 ESD, using prallethrin as a representative active substance. Indoor and outdoor 2008 emission scenarios were directly compared against their equivalent 2024 scenario, using identical insecticide product specific inputs and default inputs for all other fields as appropriate (as relevant for the original and updated ESDs, accordingly). Results of these direct comparisons showed that in numerous instances, when comparing like-for-like, calculated emissions are considerably higher for indoor scenarios when based on the updated ESD. In contrast, like-for-like comparison of outdoor scenarios show lower indirect (via wastewater for urban product uses) and direct soil (for rural product uses) emissions based on the updated ESD. However, it is noted that the updated ESD now also considers direct emissions to surface water (via rainwater discharge) for outdoor scenarios, which may potentially complicate risk assessments. Equation parameters which have undergone notable changes within the updated ESD include 'simultaneity factor' derivation and default 'line/area/volume treated with the product'. Understanding these differences is essential for ensuring consistency across future PT18 insecticide environmental exposure and risk assessments.

4.10.P-Tu390 Risk-based Approach for Regulation of Marine Pollution *Kirit Wadhia, National Oilwell Varco (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

UNCLOS. (1982). United Nations Convention on the Law of the Sea. Available at:

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4.11 Navigating Marine Pollution in a Changing Climate: Emerging Contaminants, Ecosystem Impacts, and Integrated Solutions

4.11.T-01 Advancing Ecotoxicology With Sea Stars And Brittle Stars: Current Evidence, Research Gaps And Future Directions

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Sea stars (Asteroidea) and brittle stars (Ophiuroidea) play key ecological roles but they remain underrepresented in ecotoxicological research compared with other marine invertebrates. This review examines how these taxa have been used in ecotoxicology over the past two decades, the contaminants and biological endpoints investigated and their suitability as bioindicators for environmental assessment.

A structured literature search of peer-reviewed studies published between 2000 and 2025 identified 76 relevant publications focused on exposure to anthropogenic contaminants, including metals, pharmaceuticals, microplastics, hydrocarbons, persistent organic pollutants and pesticides. Research trends, contaminant categories and biological endpoints were synthesised to evaluate current knowledge and taxonomic representation.

The most common approach was environmental monitoring, followed by laboratory exposure studies.

Combined field/laboratory designs remained scarce. Research output was uneven across different regions and focused heavily on Asteroidea, with limited attention given to Ophiuroidea despite their global distribution and suitability for toxicity testing. A wide range of endpoints were investigated, including bioaccumulation, survival, development, immune and cellular responses, behaviour, histopathology and DNA damage. Heavy metals and persistent organic pollutants were the contaminants most frequently studied, and most investigations evaluated only one endpoint under controlled laboratory conditions. Behavioural and reproductive endpoints, as well as multi-contaminant and multi-stressor designs, were underrepresented.

Overall, sea stars and brittle stars are ecologically important species that are underrepresented in marine ecotoxicology research. The development of ethical, standardised methodologies for using these organisms in toxicity assessments could enhance the ecological relevance of marine pollution research.

4.11.T-02 Presence of contaminants of emerging concern in remote areas: Arctic Ocean as a case of study

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In recent years, the presence of anthropogenic substances in remote areas has become a major concern.

Moreover, due to changes in marine patterns driven by climate change (CC), it is increasingly important to evaluate contaminants of emerging concern (CECs) in order to understand their long-range transport and persistence.

This study focuses on assessing the presence of different families of CECs in the Arctic region. Owing to Arctic amplification, climate change is more pronounced in this area, with temperatures rising three times faster than the global average. As a result, marine ecosystems in the High North are under considerable pressure. Within this context, we focused on CECs such as plastic additives, pharmaceuticals, marine toxins, and perfluoroalkyl substances (PFASs)—including legacy compounds like PFOA and PFOS, as well as persistent and mobile (PMOC) PFASs such as trifluoroacetic acid (TFA) among others.

Samples were collected during the ONE-BLUE Arctic case study sampling campaign onboard the R.V. Polarstern. Water samples were obtained throughout the entire water column using the vessel's CTD rosette sampler and a solid-phase extraction was performed onboard for CEC analysis.

The suspect screening revealed the tentative identification (confidence level 2) of several siloxanes and pharmaceuticals as well as some plastic additives and short chain PFASs. Conversely, the results from the suspect screening of marine toxins did not show the presence of these natural substances in Arctic waters. Regarding plastic additives, the compounds that have been quantified included 4-methylbenzotriazole, benzyl butyl phthalate, bis(2-ethylhexyl)phthalate, caprolactam, Citroflex 4, dibutyl phthalate, diethyl phthalate, dipropylene glycol dimethyl ether, laurilactam, tributyl phosphate, triphenyl phosphate, and tris(2-butoxyethyl)phosphate, ranging from 0.1 to 50 ng/L. In the specific case of PMOCs related to short-chain PFASs, the results showed the presence of TFA in almost all samples, at concentrations below 20 ng/L, highlighting the occurrence of this highly mobile compound in the area. Finally, the most ubiquitous compounds were the plastic additives diethyl phthalate and triethyl phosphate, as well as the short-chain TFA. These results demonstrate the presence of CECs, including PMOCs, in Arctic environments. This knowledge is highly important for elucidating the new transport patterns expected in the near future under ongoing climate change scenarios.

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4.11.T-03 Integrative Growth Inhibition and Metabolomic Alterations on Marine Microalgae *Tetraselmis suecica* Exposed to CECs

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The increasing presence of Contaminants of Emerging Concern (CECs) in marine environments poses potential risks to aquatic organisms, but their consequences on marine water bodies remain poorly understood. In this context, microalgae are key indicators of marine health, but traditional growth inhibition tests often fail to detect sublethal responses in resistant marine species.

To overcome this challenge, we evaluated *Tetraselmis suecica* as a model organism by combining growth inhibition assays with metabolomic profiling. Growth tests were optimised using OECD reference compounds (potassium dichromate and 3,5-dichlorophenol), then applied to a synthetic mixture of 14 CECs at concentrations up to 32× environmental levels. Additionally, the developed growth inhibition test was applied to 23 real marine samples collected along the Catalan Coast in the frame of the ONE-BLUE project, in parallel with suspect screening to identify chemicals potentially linked to the toxicity observed in the growth inhibition tests. Cultures were monitored spectrophotometrically for 72 h, and samples without significant growth differences were analysed for targeted and suspect metabolomics using LC-HRMS.

Results showed no growth inhibition for the CEC mixture, but metabolomic analysis revealed distinct biochemical shifts. Potassium dichromate induced overall increased levels of amino acids, including histidine, lysine, phenylalanine, and tryptophan, while CEC exposure caused marked underexpression of them. Real sample analysis revealed growth inhibition in areas near harbours and WWTP emissaries, with suspect screening detecting plastic additives and pesticides as possible contributors to toxicity.

These patterns suggest sublethal physiological effects, including oxidative stress, impaired respiration, and membrane remodelling, which may precede photosynthetic dysfunction.

Our findings confirm that metabolomic profiling provides early indicators of contaminant stress, complementing conventional lethal endpoints. This integrative approach advances marine ecotoxicology by enabling the detection of sublethal biochemical disruptions before growth effects occur, supporting improved environmental risk assessment and monitoring strategies for emerging pollutants even in real marine conditions.

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4.11.T-04 Interactive Effects of Emerging Contaminants and Climate Change Stressors: Chronic Impacts of Amitriptyline and Tributyl Phosphate on the Physiology and Behaviour of Marine Medaka (*Oryzias melastigma*)

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The increasing presence of Contaminants of Emerging Concern (CECs) in coastal ecosystems, combined with ocean warming and acidification, poses growing risks for marine organisms, yet long-term effects data remain limited. We investigated the chronic effects of two widely detected CECs on the marine medaka *Oryzias melastigma*, a robust ecotoxicological model. The individual and combined effects of chemical exposure, increased temperature (29 °C), and reduced pH (7.6) were evaluated over a 4-month exposure. Fish were exposed to amitriptyline (AMI) or tributyl phosphate (TBP) through spiked food (100 ng/g), mimicking environmentally realistic trophic transfer. The evaluation of interactive impacts of climate stress and CECs was made possible through the establishment of twelve experimental conditions combining two chemical treatments, two temperatures, two pH levels, and their interactions. Growth, metabolism, reproduction, behaviour, transcriptomics, and microbiome responses were assessed across different tissues.

Preliminary findings highlight temperature as a key driver of biological responses. Males exhibited significant growth increases at warmer temperatures, whereas females showed no clear effect. Reproductive outputs revealed an increase in egg production in females under acidic conditions, particularly with AMI exposure, suggesting a synergistic interaction between the chemical and lowered pH. However, warming consistently reduced fecundity across treatments, even when pH was lowered, indicating its role as a dominant stressor. Full interpretation will rely on ongoing analyses of F1 hatching success, parental and offspring behaviour, and transcriptomics to evaluate potential multigenerational effects and the pathways disrupted by both contaminants in combination with climate-change stressors.

Overall, the experimental framework provides a powerful approach to disentangle chemical-climate interactions and their chronic impacts on fish. By integrating behavioural, physiological, transcriptomic, and microbiome endpoints, this work will advance understanding of how marine organisms respond and adapt to co-occurring environmental stressors. The results will support improved assessment of ecological risks in future ocean scenarios shaped by pollution and climate change.

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4.11.T-05 Uncoating the Problem: Leachates From Coatings Affect Estuarine Copepods Under Climate Change Scenarios

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Antifouling coatings have been used to prevent the growth of fouling organisms on submerged vessels and structures. However, coatings undergo physical and chemical degradation, leaching the toxic biocides from their coating matrix. These leachates have been found to have hazardous effects on aquatic organisms, such as copepods, cockles, and ragworms. In parallel, climate change, particularly rising seawater temperature, exacerbates existing environmental stressors and introduces further ecological challenges. Yet, the combined effects of leachates from coatings and climate change-related stressors remain largely unexplored. The goal of this study was to investigate the combined effects of leachates from coatings and increased temperatures in the harpacticoid copepod *Nitokra spinipes*, a species with a relevant role in estuarine food webs. Paint items were produced by applying thin layers of a commercial coating onto silicone molds. Leachates were generated by immersing the paint items in a filtered 7 PSU aquatic medium for seven days, at room temperature (21 °C), in the dark. The toxicity was then assessed by exposing *N. spinipes* adults and nauplii to the leachates at 22 °C and 25 °C (+3 °C; RCP 8.5 scenario, IPCC 2021). To ensure that the organisms were responsive to the toxicants, a positive control was included using copper sulphate in acute toxicity tests on both the adults and nauplii at 22 °C. Our results showed a synergistic effect in adults resulting from combined exposure to coating leachates and higher temperature, expressed by a lower median lethal dose (LC50) at 25 °C (after 72-h, LC50_22°C = 48.8 ± 2.92% and LC50_25°C = 14.2 ± 10.00%). In contrast, the nauplii showed no significant temperature-related differences in mortality (LC50_22°C = 22.3 ± 13.08%, and LC50_25°C = 16.9 ± 1.57%), or larval development.

Therefore, the tested leachates induced significant toxicity in both life stages. Interestingly, a significant hormesis effect was observed for the larval development ratio at both temperatures, showing a positive effect from the leachates at a low dose. Our findings suggest that the predicted increase in temperature may have additional effects on the leachate's toxicity in adult copepods, and could lead to cascading effects (e.g., at the population level). These results advance our understanding of the combined effects of coating leachates and climate change on aquatic organisms.

4.11.P Navigating Marine Pollution in a Changing Climate: Emerging Contaminants, Ecosystem Impacts, and Integrated Solutions

4.11.P-We343 Warming Increases the Toxicity of Plastic Additives in the Marine Copepod *Centropages typicus*: Towards Climate-Aware Marine Pollution Risk Assessment

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Plastic additives are emerging contaminants in marine ecosystems, yet their combined effects with climate-related stressors remain poorly understood. Copepods, as key zooplankton components of marine food webs, are highly sensitive to environmental changes, and their vulnerability to pollution under warming conditions may have far-reaching ecological consequences.

This study investigated the individual and combined effects of five plastic additives: bisphenol A; dibutyl phthalate; 3,3',5,5'-tetrabromobisphenol A; triphenyl phosphate; and 2-ethylhexyl diphenyl phosphate, on the marine copepod *Centropages typicus* under two temperature regimes representing ambient (16°C) and heatwave (22°C) conditions. The goals were: 1) to determine acute toxicity thresholds (LC50 values), 2) evaluate how elevated temperature modifies toxicity, and 3) assess effects of chemical mixtures and biomarker responses to investigate sublethal stress responses.

Adult copepods were exposed in multi-well plates to the five plastic additives. Additionally, different mixtures were tested: two mixtures containing an equitoxic concentration corresponding to the total LC50 (LC10 of each additive), 2) a sublethal LC15 mixture (LC3 of each compound), and 3) a mixture at an environmentally relevant concentration. Biomarker response was analysed in the LC15 mixture to investigate sublethal physiological effects.

Results showed that increased temperature consistently enhanced the toxicity of organophosphorus compounds and dibutyl phthalate, lowering their LC50 value and revealing that thermal stress can worsen the effects of plastic-associated chemicals. The exposure to the chemical mix caused higher mortality than predicted from single-compound exposures. This may indicate synergistic interactions, with its effects amplified under heatwave conditions. Moreover, the environmental concentrations under heatwave condition caused significantly higher mortality. Exposure to the LC15 mixture induced significant biomarker responses.

These findings show that rising ocean temperatures may exacerbate the risks posed by environmental chemical mixtures associated with plastics. By considering multiple stressors and biological endpoints, this work contributes to a better understanding of contaminant effects in a changing ocean. It also contributes to realistic, temperature-inclusive approaches for environmental risk assessment and management which are essential to safeguard marine ecosystems.

4.11.P-We344 Microplastic Contamination In Deep-Sea Invertebrates From The Central Mediterranean Sea

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Microplastics are recognised as an emerging contaminant in marine ecosystems, but information on their presence in deep-sea habitats remains limited. This study addresses this knowledge gap by examining microplastic occurrence in 35 specimens representing nine species of deep-sea invertebrate collected from the Gulf of Orosei on the east coast of Sardinia, Italy. The aim was to assess the extent of contamination and to explore whether ecological traits influence microplastic accumulation patterns.

Organisms belonging to three major invertebrate groups (i.e., Arthropoda, Echinodermata and Mollusca) were sampled at depths between 250 and 450 metres. After laboratory processing to isolate and characterise microplastic particles, the polymer types and particle shapes were identified using Fourier Transform Infrared

(FTIR) spectroscopy. Statistical analyses were employed to evaluate similarities and differences among species. Microplastics were found in nearly all specimens, with a total of 105 particles recorded across the species sampled. Filaments and fragments were the most common shapes, and particles were primarily composed of polyethylene, polypropylene and polyethylene terephthalate. Regression analyses revealed a significant positive correlation between organism weight and microplastic abundance in Echinodermata, suggesting that larger individuals accumulate more particles. Cluster analysis revealed that contamination patterns were more closely aligned with ecological behaviours, such as feeding strategy, than with taxonomic relationships. These findings provide the first evidence of microplastic contamination in multiple deep-sea invertebrate species from the Gulf of Orosei. The results emphasise the importance of species-specific ecological characteristics in shaping microplastic exposure and accumulation in deep-sea environments.

4.11.P-We345 Plastic Meets Climate: Nitokra spinipes in the Face of Dual Challenge

Juliette Grandjean¹, Jana Asselman² and Ana Isabel Catarino¹, (1)*Flanders Marine Institute (VLIZ), Belgium*, (2)*Blue Growth Research Lab (BGRL), Ghent University, Belgium*

In estuarine waters, organisms are subject to multiple stressors in the environment, including contaminants of emerging concern, such as microplastics, and fluctuating physicochemical parameters due to tidal and freshwater inputs. However, due to global change, physicochemical parameters are predicted to be shifted towards new extremes in estuaries. Additionally, estuaries are considered reservoirs of increasing plastic pollution, but the assessment of the combined effects of these stressors on primary benthic consumers is lacking. Therefore, the goal of this work is to assess the combined effects of multiple stressors (temperature, salinity, microplastics) on benthic primary consumers, on both the larval and adult stages. First, the stressors (salinity, temperature, microplastics) will be tested alone through acute toxicity tests on both stages, following standard protocols, to estimate the median lethal dose (LD50). The microplastic experiments will focus on the particle's effects. Effects of the leaching chemicals from the polymer matrix will be excluded by using a dialysis test with adult animals. Based on effect data for each stressor, three carefully selected combinations (from a fractional factorial experiment) will represent a best-case, an intermediate, and a worst-case scenario, with overall toxicity levels approaching half of the LD50. These combinations will be tested over single life stages but also across multiple generations (three generations) to obtain information not only on their toxicity but also on their sub-lethal effects (e.g., oxygen consumption rate, reproduction rate and success). Overall, this study will provide a comprehensive assessment of how multiple stressors interact to affect benthic primary consumers throughout their life cycle, thereby contributing to a better understanding of their vulnerability and resilience.

4.11.P-We346 Assessing Marine Ecotoxicity of Hazardous Constituents in Oilfield Produced Water

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Produced water (PW) is the primary wastewater generated during oil and gas exploration, and many of its constituents can damage the aquatic environment. This study evaluated various substances found in PW by assessing acute toxicity using *Vibrio fischeri* and chronic toxicity with two species of sea urchins collected in Brazil, *Arbacia lixula* and *Echinometra lucunter*. The inorganic substances assessed included nitrogen, ammonia, and several metals, such as zinc, cadmium, copper, lead, iron, and manganese. The organic substances evaluated were ethylbenzene, o-xylene, phenol, o-cresol, p-cresol, and some carboxylic acids, including cyclohexane carboxylic acid, methyl pentanoic acid, cyclohexane pentanoic acid, cyclohexane acetic acid, and pentane carboxylic acid. The acute and chronic toxicity assessments were conducted in accordance with Brazilian standards, specifically ABNT 15411:2021 and ABNT 15350:2020, respectively. Ammonia nitrogen exhibited high chronic toxicity, particularly to *E. lucunter*, with a no-observed-effect concentration (NOEC) of 0.08 mg NH₃/L. This substance is a significant contributor to PW toxicity due to its generally high concentrations. The most toxic metals evaluated for all organisms tested (with NOEC values equal to or less than 1 mg/L) were iron and zinc, which are commonly found at elevated levels in produced water compared to seawater. Copper was also highly toxic to marine organisms despite its low PW concentration (<0.005 mg/L). All organic substances tested were highly toxic to the evaluated organisms; however, chronic toxicity assessments with sea urchins indicated greater sensitivity for most organic compounds than acute toxicity evaluations. Among the carboxylic acids, methyl pentanoic acid was the least toxic to *V. fischeri* (EC20 of 783.5 mg/L), but it was one of the most toxic to both sea urchin species, with a NOEC of 0.05 mg/L. Conversely, cyclohexane pentanoic acid was found to be more toxic to *V. fischeri* (EC20 of 0.787 mg/L) than to *A. lixula* (NOEC of 25 mg/L) and *E. lucunter* (NOEC of 3.12 mg/L). Naphthenic acids are considered one of the most toxic components of PW, and their hazardous presence, along with other components such as nitrogen,

ammonia, metals, and other toxic organic substances, needs attention and monitoring to prevent their release into the environment.

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4.11.P-We347 Comparing Acute Freshwater and Marine Toxicity Data to Inform the Potential Use of REACH Freshwater Data in the Harmonised Mandatory Control System (HMCS) for Offshore Oil and Gas Production Chemicals

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Over the past decade, various aspects of the Harmonised Mandatory Control Scheme (HMCS) under the Oslo-Paris Commission (OSPAR) have been updated to align with EU chemicals legislation. These updates include substance restrictions, authorisations and exemptions under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation and Biocidal Products Regulation (BPR). Currently, harmonisation with assessment factors (AFs) under REACH for the derivation of Predicted No Effect Concentrations (PNECs) is under consideration, which would allow greater use of freshwater data in the HMCS. This would be beneficial in reducing new testing and improving animal welfare if freshwater data are already available through REACH. However, the derivation of marine PNECs under REACH guidance use relatively high assessment factors (generally 10x those applied in the freshwater assessment), which may result in considerably lower PNECs than would be derived under the HMCS.

To assess whether freshwater and marine data for offshore chemicals can be used interchangeably, we have compared freshwater and marine sensitivities for fish, invertebrates and algae based on a literature review, covering various types of chemicals, and a de novo analysis of freshwater and marine acute toxicity data for offshore oil and gas production chemicals. The ratio of freshwater to saltwater (FW:SW) toxicity is frequently reported in literature as a parameter to compare sensitivities, with a ratio within a factor of 10 proposed by some papers to indicate equivalent sensitivity between freshwater and marine species. Therefore, this parameter was applied throughout this assessment to provide a consistent comparison of freshwater and marine sensitivities in both the literature review and de novo analysis. Statistical analyses of freshwater and marine datasets were also conducted.

This poster will present the findings of this analysis and discuss the suitability of harmonisation with REACH assessment factors for PNEC derivation under the HMCS. Consideration will be given to the applicability of freshwater toxicity data for marine environmental risk assessment.

4.11.P-We348 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?

Reference

OSPAR (2012), Guidelines in support of Recommendation 2012/5 for a Risk-based Approach to the Management of Produced Water Discharges from Offshore Installations. Oslo-Paris Convention for the Protection of the Marine Environment of the North-East Atlantic. Agreement: 2012-7. London, UK.

4.11.P-We349 PEARL Project: Integrated Assessment of Environmental Fate and Ecotoxicological Risk of Chemical Emissions from Offshore Wind Farms

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The deployment of offshore wind energy raises environmental concerns, particularly regarding chronic chemical emissions that may affect ecological quality within and around Offshore Wind Farm (OWF) areas. Among these emissions, those related to cathodic protection systems using galvanic anodes (GACP) and impressed current (ICCP) are of particular concern. Additionally, emerging floating technologies may introduce new environmental concerns, such as the potential chemical pressure from synthetic mooring lines, which should be anticipated prior to large-scale deployment.

The PEARL project adopts an integrative and multidisciplinary approach to define the chemical risks associated with chronic releases from GACP and ICCP systems across all environmental compartments (water, sediments, and biota) potentially impacted by the development of offshore wind projects in the English Channel and North Sea, as well as along the Atlantic and Mediterranean coasts.

The project combines laboratory experiments, in situ monitoring, and hydro-sedimentary modelling to investigate the behaviour, fate, and local accumulation of metallic elements released by GACP. Modelling will integrate current and future OWFs up to 2050 with other anthropogenic activities and potential natural sources. In parallel, the project will assess environmental pressures generated by ICCP by determining chlorine production rates under different configurations and developing analytical methods to detect chlorination by-products in marine environments.

Ecotoxicological investigations will combine laboratory and in situ ecotoxicological approaches, evaluating model species responses to GACP and ICCP exposure, together with biomonitoring in OWF. Additionally, modelling work will build on these results to assess concentrations of elements originating from GACP within the trophic web around a case study area in Fécamp OWF.

Finally, PEARL will also address chemical pressures from the ageing and degradation of synthetic mooring lines (polyester, polyamide), implementing laboratory ageing protocols to characterize element releases and assess potential environmental impacts.

By integrating experimental, field, and modelling approaches, PEARL will provide a scientific basis for OWF risk assessment, supporting sustainable marine energy and informing regulations.

4.11.P-We350 The risks of multiple human activities to benthic biodiversity disturbance in the Belgian Part of the North Sea: quantitative assessment using an adapted InVEST model

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Marine ecosystems increasingly face cumulative pressures from multiple human activities that act as interacting stressors on biodiversity and the ecosystem services it underpins. In the Belgian Part of the North Sea (BPNS), physical disturbance from bottom-contact fishing, sediment extraction, dredging, disposal of dredged material, and the rapid expansion of offshore wind energy contribute to shifting seafloor conditions and altered benthic community structure. Despite extensive monitoring, current cumulative risk assessments often rely on qualitative scoring and lack empirical grounding, limiting their relevance for evidence-based marine spatial planning. Here, we present a data-driven adaptation of the InVEST Habitat Risk Assessment (HaRA) model that integrates high-resolution macrobenthos monitoring with spatially explicit activity-intensity layers to quantify biodiversity disturbance risk under realistic multi-stressor exposure. Generic categorical scoring criteria are replaced by continuous, empirically derived exposure–consequence relationships based on five biodiversity indicators, enabling intercomparable, decision-ready risk estimates. Applied to the BPNS, the model identifies

bottom-contact fishing as the dominant driver of benthic biodiversity disturbance, while extraction and dredging produce strong but localized effects. Scenario analysis indicates that planned offshore-wind expansion will increase local biodiversity-disturbance risk within new concession zones but is expected to cause only minor basin-wide changes, reflecting prior spatial planning was successful in selecting locations where human development and ecological risks are well balanced. By empirically grounding risk estimates and capturing interactions among multiple marine stressors, this adapted HaRA framework provides an integrated, transparent tool to support marine spatial planning, conservation prioritization, and transparent trade-off analysis among competing uses.

4.11.P-We351 The origin of sediment organic carbon influences hydrophobic organic pollutant dynamics in Arctic shelf sediments

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The fate of hydrophobic organic pollutants in the marine environment is largely controlled by organic carbon (OC) cycling processes. The Arctic is warming three times as fast as the global average and climate change is profoundly altering OC fluxes due to e.g. intensified permafrost thaw, enhanced river runoff and shifting primary production dynamics. As a consequence, remote Arctic shelf sediments serve as an ideal receptor for quantitative and empirical assessments of the impact of OC quantity and quality on pollutant fate. Here, we compiled a database of congener-specific polychlorinated biphenyl (PCB) concentrations (514 entries, 85 sites) in surface sediment of four Eurasian Arctic shelves via integration of new measurements and literature data. Total organic carbon content and isotopic data were retrieved or predicted from CASCADE (The Circum-Arctic Sediment CARbon DatabasE) to assess the relative contribution of terrestrial and marine OC in the sediment. In order to reduce variability in water-phase concentrations, influences from longitude and depositional year were corrected. And samples were grouped into the Barents Sea and the Laptev and East Siberian Seas, due to differences in congener profiles. The resulting variability in concentration was used to infer sediment absorption propensity of PCBs. In the Barents Sea, we found that marine OC resulted in 5-15 times (mean: 11 times) higher concentrations (in ng-PCB/g-OC), compared to terrestrial OC. In the Laptev and East Siberian Seas, the positive effect of marine OC on PCB sequestration was the most pronounced for higher chlorinated PCB congeners. No statistical differences were found between terrestrial and marine OC for tri- and tetra-PCBs, but absorption propensities of marine OC were 6-7 times higher for penta-PCB and 16-22 times for hexa-PCB. Based on these results, PCB sequestration in Arctic shelf sediments was predicted under three future scenarios for climate change using an observational dataset of terrestrial inputs and marine OC fluxes derived from the global 'state-of-the-art' ocean and sea ice biogeochemistry model FESOM2.1-REcoM3.

4.11.P-We352 Cleaning Sediments and Restoring Ecosystems – Evolving Towards an Adaptive Risk Assessment and Management Framework

Robert K Johnston, Applied Ecological Solutions

Cleanup at many large contaminated sediment sites can be very costly, time consuming, and ineffective if larger scale pressures and ecological processes are not adequately addressed by the remedial design. A holistic approach is ideal—one that integrates remediation with restoration to maximize synergies, reduce costs and environmental impacts, and promote long-term sustainability. Sustainable remediation strategies should be informed not only by considerations of regulatory compliance but also by stakeholder goals, values and expectations. Traditionally, remediation efforts have focused on mass removal or reducing exposure to sediment-associated contaminants to address human health and ecological risks. However, evaluating the success of such remedies has proven challenging due to the complexity of ecosystem interactions, the lack of comprehensive monitoring data, and the need to align cleanup objectives with ecosystem recovery.

Additionally, sediments are linked to processes occurring within the watershed making them susceptible to, and potential drivers of, chemical, physical, and biological stressors from larger scale social and economic pressures, climate change, and other disturbances associated with the Anthropocene. Drawing on the results of case studies, key concepts and lessons learned to better integrate sediment cleanup and ecosystem recovery goals are discussed. By integrating risk assessment and adaptive management into a unified Adaptive Risk Assessment and Management framework—incorporating the Plan-Do-Act-Check-Learn adaptive management cycle alongside an iterative risk assessment process—organizations can enhance risk management decisions, foster continuous stakeholder engagement, and ultimately improve both cleanup and restoration outcomes. This approach ensures flexibility, promotes ongoing learning, and aligns remediation strategies with evolving ecological and sustainability goals.

Disclaimer/Disclosure:

This work is the view of the author and does represent the views of any government agency.

4.11.P-We353 *Ostreopsis cf. ovata* and benthic invertebrates in the Mediterranean Sea: an ecotoxicological study

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Harmful algal blooms (HABs) are proliferations of microalgae affecting environmental, animal, or human health. Benthic toxin-producing microalgae present emerging risks in Europe, notably the dinoflagellate *Ostreopsis cf. ovata*, which produces toxins of the ovatoxin (OVTX) group. Blooms of this species pose potential health risks to humans through direct exposure and seafood contamination, while also being linked to mass mortalities of sea urchins. However, the broader ecological consequences of these blooms and the pathways of toxin transfer through food webs remain poorly understood.

This study investigates the impact of *Ostreopsis cf. ovata* and its associated toxins on benthic invertebrate communities and evaluates the potential role of these organisms as vectors of biotoxin transfer through coastal food webs. To address this, we conducted a field study along the French Riviera during summer 2024, including sampling of both *O. cf. ovata* and benthic macrofauna. In total, 12,596 invertebrates from 100 genera were collected and identified for ecological and toxin analysis.

Invertebrate species identification and enumeration indicated changes in benthic invertebrate community composition correlated with the abundance of *Ostreopsis cf. ovata*, with certain taxa exhibiting declines while other increased in abundance. Toxin analysis using neuroblastoma cell-based assay combined with LC-MS/MS quantification of OVTX analogues (OVTX-a to -e) indicated that some of the most abundant taxa exhibited the highest OVTX concentrations, reaching up to 50 ng total OVTX/g in some amphipods. This suggests that certain invertebrate taxa may be resistant to *Ostreopsis* toxins, allowing OVTX to accumulate within their tissues at high concentrations.

By integrating ecological, toxicological, and public health perspectives, this study demonstrates that *Ostreopsis* blooms alter benthic community composition, which may promote toxin transfer through trophic chains and thereby pose a threat to seafood safety. These findings underscore the need for reinforced monitoring and adaptive management strategies to address the One Health consequences of HABs in the context of climate change and increasing anthropogenic pressures on Mediterranean coastal ecosystems.

4.11.P-We354 Effects of Rising Temperatures on Oxidative Stress Biomarkers in the Mussel *Mytilus galloprovincialis*

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The Mediterranean coastline is widely recognized as a highly vulnerable environment, showing strong sensitivity to the effects of climate change. Rising sea temperatures are expected to have a significant impact on the physiological and metabolic responses of many marine organisms. Among them, mussels, particularly *Mytilus galloprovincialis*, represent a valuable model species for both aquaculture and ecological or ecotoxicological research. They are widely used as sentinel organisms for monitoring marine pollution and have proven to be highly reliable bioindicators under controlled laboratory conditions.

To obtain a more comprehensive understanding of the effects of temperature on *M. galloprovincialis*, we tested five different thermal conditions. A total of 500 mussels were used in the experiment, with 100 individuals assigned to each temperature. For every thermal condition, the 100 mussels were distributed into four replicate aquaria, each containing 25 individuals. Each aquarium held 20 L of water and was equipped with continuous aeration. The water temperatures were set to 17 °C (control), 22 °C, 24 °C, 26 °C, and 30 °C. Mussels were exposed to these conditions for 30 days. At each temperature, 10 individuals were randomly sampled at 12 and

24 hours, and at 3, 5, 10, 15, 20, and 30 days. Water parameters remained constant throughout the experiment. At each sampling time, mussels were sampled and the tissues were stored at -80°C for subsequent biochemical analyses. The tissues were analysed using a suite of enzymatic oxidative stress biomarkers to assess the physiological stress experienced by the organisms under simulated climate-change conditions. The results highlight the influence of temperature on the enzymatic stress of mussels, with distinct trends observed across the analysed tissues. These findings are particularly relevant in the context of climate change, as organisms already experiencing stress may exhibit a heightened sensitivity to additional stressors, such as pollutants or bacterial and parasitic diseases. It is noteworthy that some of these stressors may be intensified by rising temperatures.

Disclaimer/Disclosure:

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4.11.P-We355 Power Under Pressure: Cellular Energy Allocation and Glutathione S-Transferase Reveal Bivalve Stress from Exposure to Natural Radioactivity

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Although the discharge of industrially derived Naturally Occurring Radioactive Materials (NORM) is a major source of radiological and chemical contaminants in the marine environment, their ecotoxicological effects on marine organisms remain poorly understood. We conducted Cellular Energy Allocation (CEA) and glutathione S-transferase (GST) assays as indicators of physiological stress in three edible bivalve species, *Cresstoderma edule*, *Mytilus edulis* and *Magallana gigas*, after laboratory-controlled exposures to polonium-210 (210Po) and lead-210 (210Pb), two of the most radiotoxic naturally occurring elements, via seawater and spiked algae mix. Total CEA was calculated as the ratio of energy budget (in the form of lipid, carbohydrate and protein) to energy consumption (Electron Transport System) to determine the overall impact on tissue or organisms' energy expenditure. GST activity was quantified in tissue samples as a key biomarker of detoxification. At the end of the 14-day exposure, *C.edule*, had the highest mean 210Po activity concentration followed by *M. edulis* and *M. gigas* (wet weight concentrations). Significant deviations from control group values in total CEA were observed in some exposure groups, namely *M. gigas* digestive and gill tissue samples with decreased values, and *C.edule* (whole body) with an increase in total CEA. Analysis of individual energy budget components revealed no consistent trends across species or tissue types. Conversely, GST levels significantly decreased in *C. edule* after exposure, and in *M. Edulis* and *M. gigas* were significantly higher, particularly in the digestive gland. Exposure to 210Po and 210Pb was shown to elicit a discernible stress response in the physiological energy budgets of two of the three selected bivalve species and induced measurable changes in detoxification biomarkers across all species examined. The radiological hazard and ecological risk from chronic exposure to legacy NORM discharges in the marine environment are measured using specific metrics and traditionally considered in isolation from other health hazards and risks. Ecotoxicological endpoints can help bridge this knowledge gap and present an innovative opportunity for evaluating such risks. The need for such approaches is heightened by the potential change in uptake, speciation and mobilisation that current environmental stressors (climate change and acidification) might pose to industrially derived NORM.

4.11.P-We356 Cellular Responses in Haemocytes Induced by Exposure to Chemicals of Emerging Concern

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The increasing use and production of chemicals has led to a rise in the number of contaminants of emerging concern (CECs) in the marine environment. Assessing and prioritizing compounds with insufficiently characterized adverse effects is crucial to ensure environmental and ecological protection. In this study, cytotoxic and early-warning effects in haemocyte responses of *Mytilus edulis* after single- and mixture exposure of 12 CECs plus one transformation product using flow cytometry. The results demonstrated that CECs modulated several cellular parameters, including cell metabolism, reactive oxygen species (ROS) production, intracellular calcium levels, mitochondrial membrane potential (MMP) and multi-xenobiotic resistance (MXR) activity. However, cytotoxicity was predominantly observed in granulocytes exposed to highest concentration of 6-PPD, 6-PPD-quinone, 2-ethylhexyl diphenyl phosphate, triclocarban, citalopram, and octocrylene. Based on these findings, NOEC, EC10, and EC25 values were extrapolated for the respective compounds and applied to

design a mixture experiment (6 CECs × 3 levels; Definitive Screening Design model). The results identified 6-PPD as the primary driver of cellular alterations, affecting 4 of the 6 parameters in granulocytes and 3 of the 6 in hyalinocytes. In granulocytes, MMP was exclusively affected by citalopram, which also contributed to 4 of the 6 altered endpoints in granulocytes and 2 of the 6 in hyalinocytes. Moreover, 6-PPD-quinone was identified as the sole driver of intracellular calcium disruption in hyalinocytes, while triclocarban contributed to both cytotoxicity and ROS production in granulocytes. Octocrylene was associated with increased ROS generation in both haemocyte subpopulations. Finally, EDHPHP, which did not significantly affect early-warning biomarkers when tested individually, was shown to drive ROS production and MXR activity in the mixture experiment. Compounds of emerging concern (CECs) were found to modulate a wide range of cellular parameters in *Mytilus edulis* haemocytes. The cellular responses were strongly influenced by intrinsic differences between haemocyte subpopulations. Mixture exposures demonstrated that chemical interactions can substantially modify the toxicity profiles of CECs. Overall, the study highlights the complexity of CEC-induced cellular stress and reinforces the value of haemocyte-based biomarkers for detecting early ecotoxicological effects.

4.11.P-We357 Interactive effects of citalopram and climate stressors on sea urchin embryo development

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Marine ecosystems are increasingly exposed to combined pressures from chemical pollutants, plastic debris, and climate change, with the presence of the pharmaceutical Citalopram (CIT) in coastal waters coinciding with projected ocean warming (OW) and acidification (OA). Using the sea urchin *Paracentrotus lividus* as a robust model, this study assessed how CIT toxicity is modulated under factorial combinations of these climate stressors during early embryo development (Control: 20 °C, pH 8.0; OW: +4°C; OA: pH 7.6; OW+OA). CIT alone significantly decreased growth, while acidification alone caused no effect but revealed a synergistic toxicity with CIT, resulting in stronger growth inhibition. Conversely, ocean warming, whether alone or combined with OA, masked the inhibitory effect of CIT on growth, leading to a slight increase. However, morphometric analysis demonstrated a consistent detrimental effect of warming, as embryo width significantly decreased across all treatments involving elevated temperature, while width remained stable under OA, CIT, or their combination without warming. These findings highlight that acidification is the main driver of synergistic toxicity concerning growth, whereas warming acts as a secondary stressor specifically compromising embryo width, underscoring the necessity of integrating multi-stressor interactions into ecological risk assessments for future ocean scenarios.

Disclaimer/Disclosure:

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4.11.P-We358 Establishment of Baseline Total Hemocyte Count (THC) in the Marine Amphipod *Parhyale hawaiiensis*

Natália de Farias, Ana Julia Gotto, Andrey Majarowsky, Jéssica Cardoso and Gisela Umbuzeiro, Universidade Estadual de Campinas, Brazil

Parhyale hawaiiensis is a marine, epibenthic, circumtropical organism that has been increasingly used across different research fields, ranging from toxicity studies to evolutionary developmental biology. Among these areas, immunotoxicology has gained particular prominence due to growing concerns about the immune health of aquatic organisms continuously exposed to many environmental contaminants. There is an urgent need to better understand how the immune system of these organisms, especially marine invertebrates, responds to contaminant exposure. Hemocytes play a central role in the immune response of these organisms, being responsible for key defense functions such as phagocytosis, encapsulation, coagulation, and the production of reactive oxygen species and antimicrobial peptides. The Total Hemocyte Count (THC) represents one of the most accessible and informative biomarkers for assessing immunological status. Although *P. hawaiiensis* has already been established as a model species in ecotoxicology, its application in immunotoxicological studies still requires methodological refinement. The aim of this study was to establish reference hemocyte values for male and female *P. hawaiiensis*, providing basis for developing THC assay protocol. Two age groups, 5 and 8 months old were used, each comprising 100 organisms (50 males and 50 females). Hemolymph was collected from the dorsal region between the 1st and 2nd segments, transferred to a 4% paraformaldehyde solution, and

subsequently placed in a Neubauer chamber for counting under an inverted microscope. For 5-month-old organisms, females exhibited a mean ($\pm$ SD) hemocyte count of $10,029 \pm 10,727$ hemocytes per individual, while males showed $12,389 \pm 9,890$ hemocytes per individual. In 8-month-old organisms, females exhibited $14,395 \pm 12,790$ hemocytes per individual, and males $14,240 \pm 11,705$ hemocytes per individual. Both males and females at 8 months showed a slight increase in hemocyte abundance compared to those at 5 months. No statistically significant differences in THC were detected between males and females at either age. This study establishes reliable baseline THC values for male and female *P. hawaiiensis* at two developmental stages. Further research is required to determine whether THC can serve as a robust endpoint in ecotoxicological studies when *P. hawaiiensis* are exposed to compounds with known immunomodulatory activity.

Disclaimer/Disclosure:

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4.11.P-We359 Acute Toxicity Testing in the Marine Medaka (*Oryzias melastigma*): Protocol Adaptation and Refinement

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Accurate assessment of chemical toxicity in marine environments is essential for effective ecosystem protection. Current regulatory tests are predominantly designed for freshwater species, which can lead to inaccurate extrapolations when applied to marine organisms. To address this gap, we developed a high-throughput fish embryo acute toxicity protocol using the marine medaka (*Oryzias melastigma*), a species well-suited for laboratory testing due to its small size, short lifecycle, non-seasonal reproduction, transparent chorion, and sexual dimorphism.

Embryos up to 12 days post fertilization were assessed for key endpoints indicative of acute toxicity, including hatching, mortality, malformations, heart rate, and larval photo-motor response. Experiments explored combinations of intrinsic factors (age, developmental stage) and extrinsic factors (incubation temperature, exposure vessel, measurement day) to optimize protocol reproducibility and endpoint sensitivity. Larval development and behavioural performance were most consistent at 28°C, enabling the evaluation of all endpoints within the experimental period and providing robust detection of acute toxic effects.

The optimized protocol is currently being validated using triclosan as a positive control to generate reproducible data on method responsiveness and support inter-laboratory standardization. This work provides an experimentally grounded framework for acute toxicity testing in marine fish embryos, offering a reliable, ethical, and high-throughput approach that enhances the relevance and accuracy of marine ecotoxicological assessments.

Disclaimer/Disclosure:

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4.11.P-We360 Abidjan Convention and the Chemical Management Strategy

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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.

The Convention on Cooperation for the Protection, Management and Development of the Marine Environment and Coastal Areas of the West, Central and South Africa Region (Abidjan Convention) has been instigated with the rationale of integrating a concerted management of regional ecosystems. Pertaining to the Convention, the Malabo Protocol provides Contracting Parties of the region with a common framework to better supervise the development of these activities and ensure their compatibility with the protection of the marine environment. This development came in response to the accelerated development of offshore oil and gas activities in the region. The implementation plan fundamentally requires a clear understanding of a viable strategy, taking into account the elements and aspects that currently exist.

The objective of this conveyance is to examine the pertinent considerations of adaptation of chemical management strategy from a global perspective. The goals and plan to align with existing international standards will be assessed. Implications of capacity and infrastructure to achieve defined standards and the need for scientific and technical co-operation to facilitate progress will be analysed. Challenges and concerns focused on categorisation of chemicals and ecotoxicological testing will be addressed, as well as monitoring programmes and implementation procedures and overall objectives.

In essence the existing challenges of the Malabo Protocol will be highlighted, and potential scope for prudent course of action to advance the mission of the Abidjan Convention will be portrayed. This fulfils the aim to “Protect, Conserve and Develop the Abidjan Convention Area and its Resources for the Benefit and Well-being of its People.”

4.12.P Next Generation Environmental Risk Assessment: Incorporating NAMs, AI, and Big Data for Real World Decisions

4.12.P-Th171 Next-Generation Risk Assessment for Reproductive and Developmental Health Implications of Agrochemicals

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The expanding use of agrochemicals has raised concern over their reproductive and developmental health implications. However, the diversity of compounds and complexity of real-world exposure patterns limit the capability of traditional toxicology to generate reliable human health risk assessments. Next-Generation Risk Assessment (NGRA) addresses these limitations by integrating exposure science, mechanistic understanding, and NAMs within an evidence-based, decision-oriented framework. In line with this paradigm, we developed an integrated exposomics platform to characterize exposure profiles of agrochemicals in human populations and to continuously identify emerging contaminants in environmental and biological samples along with their potential health implications. By combining exposomic data with deep-learning models, we simulate complex real-world exposure patterns and identify potential health impacts, enabling predictive prioritization of high-exposure-risk chemicals. Furthermore, advanced high-throughput systems, including organoids and organ-on-chip technologies, allow us to sensitively detect biological responses induced by agrochemicals during critical reproductive and developmental windows, and to elucidate their mechanisms of action on reproductive function, early developmental processes, and key regulatory pathways. Finally, we established the Targeted Risk Assessment of Environmental Chemicals (TRAEC) strategy, which emphasizes multi-source evidence integration as core principles of NGRA. Using this framework, atrazine demonstrated a higher composite neurodevelopmental toxicity score (8.70) compared with pyridaben (7.64), indicating differential levels of concern. This framework provides an operational approach for hazard identification, prioritization, and regulatory decision-making for agrochemicals, and establishes a systematic foundation for future research on chemical exposure and public health.

4.12.P-Th172 Extending the Health Impact Pathway Approach to Environmental Risk Assessment. Challenges and Opportunities

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Next Generation Risk Assessment (NGRA) frameworks, based on NAMs and Adverse Outcome Pathways (AOP) are emerging. However, in most cases the approach is to accommodate the new, usually mechanistic based, information, to the current risk assessment paradigm, proposing NAM-based Points of Departure (PoD). Setting a PoD based on no effects at initial Key Events is a protective approach, the difficulty appears when trying to quantify the progression towards adversity. Risk assessment is by definition an identification of likelihoods, not certainties, and this aspect is frequently missed in PoD-based approaches. Quantitative AOP focuses on direct toxic effects. Indirect consequences, including stress adaptation are not covered, despite the evidence that in many cases indirect effects are leading the main environmental impacts.

For Human Health, we have proposed a new concept, Health Impact Pathways (HIP), using AOP networks for supporting the identification of intermediate events linked to physiological consequences with health relevance. Here we are exploring the applicability of this approach to environmental NGRA. HIPs consider that the

induction of a KE already represent a chemical stress, the individual may avoid progression towards adversity through physiological and/or structural adaptations. The conceptual change vs. AOP frameworks is the consideration that these changes, even not progressing towards the specific adverse outcome, increase the susceptibility and, as a consequence, the probability of the individual to suffer direct or indirect health effects. The proposed extrapolation to population level consequences is based on the consideration of the potential effects of the chemical stress associated to intermediate KE on key population dynamic drivers; that should be adapted to the biological and ecological characteristics of the different species and traits. A main advantage of this approach is the consideration of morbidity associated changes for modelling the expected impact of chemical exposure on the population dynamics, including behavioural effects associated to neurotoxicity drivers, increased susceptibility to infection diseases linked to immunotoxicity drivers, among others. The implementation is probabilistic should be adapted to the characteristics of the focal species. AI is proposed for facilitation the data extraction and analysis of interactions.

4.12.P-Th173 The Source To Outcome Pathway (STOP) – Next Generation Risk Assessment (NGRA) put into practice

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Advancing environmental risk assessment requires a paradigm shift from traditional single-chemical evaluations to more holistic approaches capable of addressing complex, real-world chemical exposures. Next Generation Risk Assessment (NGRA) promotes this transition by integrating diverse data sources and mechanistic understanding to inform both single and cumulative chemical risk assessments. Central to this evolution is the Source-To-Outcome Pathway (STOP) framework, which combines the Aggregated Exposure Pathway (AEP) and Adverse Outcome Pathway (AOP) concepts. AEP structures information on chemical use, fate, and exposure, while AOPs link molecular initiating events to adverse biological outcomes. Together, they support a more transparent and mechanistically informed weight-of-evidence approach.

As bioassay data spanning different levels of biological organisation proliferate, frameworks like STOP will become essential to manage and contextualize disparate data. This work illustrates the use of STOP in NGRA through a case study involving priority pollutants and emerging contaminants, demonstrating how qualitative descriptions and quantitative assessments can be combined for more wholistic assessment. It will also introduce a Graphical User Interface, the Source-to-Outcome Predictor (STOPredictor; <https://www.niva.no/source-to-outcome-predictor>) that aligns exposure information with effects thresholds to predict risk of ecologically relevant mixtures of pollutants.

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4.12.P-Th174 Integrated Risk Assessment of Bisphenol Compounds on Obesity Using the TRAEC Strategy and Large Language Model-Assisted Evidence Synthesis

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Bisphenol compounds are widely used environmental chemicals with increasing concern regarding their contribution to obesity, yet the strength and consistency of evidence across exposure contexts and chemical analogues remain insufficiently clarified. To address this gap, we performed a comprehensive risk assessment based on the Targeted Risk Assessment of Environmental Chemicals (TRAEC) strategy, synthesizing evidence from 39 epidemiological studies, 93 in vivo studies, and 103 in vitro studies retrieved from major scientific databases. This assessment aimed to systematically evaluate the obesogenic potential of bisphenol analogues and to integrate epidemiological findings with mechanistic data streams. Using the TRAEC scoring framework, including reliability, correlation, risk intensity, and evidence integrity scores, we found that adult exposure to bisphenol compounds confers a moderate strength of obesogenic risk overall. Among the five major analogues assessed (BPA, BPS, TBBPA, BPAF, and BPF), tetrabromobisphenol A (TBBPA) demonstrated the highest integrated obesogenic risk score (8.69), whereas bisphenol F (BPF) showed the lowest score (5.32), with epidemiological evidence for BPF exhibiting notable inconsistency at environmentally relevant exposure levels. These differentiated risk profiles reflect both structural diversity and heterogeneous biological activity across bisphenol subclasses. To support transparent and scalable evidence synthesis, we further established a large-

language-model assisted systematic review framework, incorporating ChatGPT-5.1, DeepSeek-v3.2, Claude-4, Gemini-2.5 Pro, and Kimi (K2 Thinking). This framework enabled automated long-text extraction, mechanistic mapping, and cross-study scoring, demonstrating the feasibility and added value of LLMs in handling complex multi-stream evidence during environmental chemical risk assessment. Overall, our findings demonstrate that bisphenol compounds, particularly TBBPA, pose measurable obesogenic risks, while some analogues such as BPF show weaker or inconsistent associations under real-world exposure conditions. This multi-stream assessment underscores the importance of integrating epidemiological and mechanistic evidence using structured frameworks such as TRAEC, and highlights the promise of AI-assisted methodologies in advancing chemical risk assessment.

4.12.P-Th175 The MAGIC Effect Graph – An Ecotoxicological Knowledge Base

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Laboratory effect data are needed to assess environmental risks of chemicals. Databases (db), such as the US EPA ECOTOX Knowledgebase and the EFSA OpenFoodTox, collect and provide these data to the public. Modern approaches like machine learning (ML) can unlock these data to assess the potential environmental impact of the large, increasing number of manufactured chemicals, but they rely on a high data quality for robust results. For the MAGIC effect graph, we compiled six large effect dbs with different scopes (including standardized and non-standardized tests) into a single graph db (668k endpoints, 10k chemicals, 10k biological taxa) by harmonizing terminologies and deriving quality indicators based on study parameters and curation status. Data is enriched by connecting it to additional sources (e.g., the GBIF Backbone Taxonomy) and by applying ML to predict missing labels (i.e., acute, chronic). As a result, the MAGIC effect graph provides the following solutions:

1. It offers a harmonized view over six of the most important dbs for laboratory effect data. With the MAGIC effect graph, all these dbs can be queried simultaneously.
2. Additional curation of the underlying data allows for consolidation of the ecotoxicological knowledge in the source dbs. By synchronizing the terminologies of the source db, it is for the first time possible to get an overview over the available effect data without overlaps that may bias the results.
3. A normalized score (0-1) aggregates essential quality indicators to help interpret and select reported effect endpoints, e.g., in situation where different effect concentrations are reported.

We demonstrate the usefulness of the score by benchmarking four hypothetical endpoint selection scenarios (S1-4) with original, curated study results from two large-scale studies in Germany and the US, respectively. In S1 the minimum effect concentration of all available was selected, in S2 the effect concentration with the highest score, in S3 a randomly selected effect concentration, and in S4 the median effect concentration among those above a fixed score. Applying S1-4, the analysis of the two case studies was recreated and checked whether the original interpretations still hold true. Our results show that scenarios using the score generally outperform others in reenacting original results, indicating the score is a valuable indicator for informed effect data selection. The MAGIC effect graph will be made available to the public.

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4.12.P-Th176 High-Throughput Transcriptomics for Prioritization of Per- and Polyfluoroalkyl Substances in Drinking Water

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Per- and polyfluoroalkyl substances (PFAS) comprise a large class of persistent synthetic chemicals with potential adverse health effects. The increasing detection of PFAS in drinking water has raised widespread concern and motivated regulatory actions. However, toxicological data remains unavailable for most PFAS,

limiting their inclusion in large-scale hazard evaluation. High-throughput transcriptomics (HTTr), which measures genome-wide expression changes in human-relevant cell models, offers a promising tool for rapid chemical prioritization. In this study, we integrated transcriptomic points of departure (tPODs) derived from HTTr assays with drinking water occurrence data to prioritize PFAS based on their combined toxicity potentials and exposure levels. HTTr data for multiple PFAS across several human-derived cell lines were retrieved from the U.S. EPA CompTox Dashboard. Multiple quantitative approaches were used to derive tPODs for each cell line, and the resulting values were compared across methods. The Toxicological Priority Index (ToxPi) was then applied to integrate these multi-method results into a composite toxicity potential score for each PFAS. A systematic literature search and screening process was conducted to compile PFAS drinking water occurrence data across different countries and regions. A risk index approach was used to integrate the ToxPi score with the exposure concentrations to rank PFAS across geographic areas. This study proposes a conceptual framework that applies HTTr information to rank PFAS detected in drinking water, supporting monitoring and regulatory decision-making for PFAS.

4.12.P-Th178 Next-Generation Environmental Risk Assessment for Chemicals Regulated under Water Quality Standards in Korea and the European Union

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Despite continuous monitoring of water quality standard chemicals, frequent detection of emerging contaminants and limited ecotoxicological data availability have created persistent regulatory gaps in aquatic ecosystem protection. This study developed a Next-Generation Environmental Risk Assessment (NGeRA) framework to screen and prioritize water quality standard chemicals in Korea and the European Union (EU). The study focused on drinking water and national standard monitoring substances in Korea and the EU. A total of 279 chemicals from the Seoul Water Institute in Korea were included (91 regulated monitoring substances, 136 unregulated emerging contaminants, and 52 substances with insufficient toxicity data), along with 72 national water quality standard chemicals. From the EU, 73 drinking water monitoring substances and 88 water environment monitoring substances were assessed. Among these, 48 chemicals were identified as common to both Korea and the EU. These substances were evaluated across multiple toxicity endpoints using high-throughput in vitro data from ToxCast and in vivo ecotoxicological data from the ECOTOX database. To address data gaps, in-house developed machine learning models and QSAR prediction tools validated against OECD ecotoxicological test guidelines were employed.

Toxicological evidence was integrated using a Weight-of-Evidence (WoE) approach incorporating Adverse Outcome Pathway (AOP) network. Chemicals were prioritized based on data availability and predicted ecological hazard potency. Of the substances commonly regulated in both Korea and the EU, 21 were determined to have sufficient information and low ecological concern, requiring no further assessment. Conversely, 27 substances exhibited potential hazards across multiple endpoints—including acute toxicity, chronic toxicity, and endocrine disruption—and were identified as priority candidates. For these priority substances, Species Sensitivity Distribution (SSD) modeling and cross-species extrapolation were applied according to data availability and hazard profiles.

This study presents an NGeRA-based framework for assessing water substances without animal testing. By integrating multiple levels of New approach methodologies (NAMs), it supports evidence-based identification of priority chemicals and informs policies for regulatory refinement, prioritization, and environmental standard updates to protect aquatic ecosystems.

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4.12.P-Th179 Assessment of Air Pollution-Related Health Risks from Stratospheric Aerosol Injection

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Global mean temperature has increased by approximately 1.5°C since the pre-industrial era. Under current climate mitigation efforts, achieving the 1.5°C and 2°C temperature goals set by the Paris Agreement is considered challenging. One of the mitigation strategies gaining attention is Stratospheric Aerosol Injection (SAI). SAI aims to lower the Earth's surface temperature by injecting aerosols into the stratosphere at an altitude

of approximately 20 km to enhance the reflection of incoming solar radiation. However, numerous uncertainties remain, including potential health impacts from aerosol particles and the risk of stratospheric ozone depletion, resulting in significant hesitation toward large-scale implementation.

The objective of this study is to quantitatively assess the health risks associated with SAI by estimating country-level increases in PM_{2.5} concentrations and the resulting health losses. The amount and locations of SO₂ injection are determined based on the SAI injection model. Increases in tropospheric PM_{2.5} from stratospheric aerosol injections are calculated using a chemical transport model. Changes in health risks associated with PM_{2.5} increases are estimated using concentration–response functions for air pollution. The additional health burden due to SAI is calculated by subtracting baseline health losses under no-SAI conditions from those under SAI-induced PM_{2.5} increases, enabling country-level analysis of health impacts.

The results indicate that SAI implementation increases PM_{2.5} concentrations, thereby increasing health losses. Moreover, because aerosol injection locations are determined based on latitude, the magnitude of additional health risks varies regionally.

Future work will incorporate all related impacts, including aircraft manufacturing and jet fuel production and consumption to provide a comprehensive assessment of SAI implementation. Comparing these impacts with the expected temperature reduction from SAI will allow for a trade-off analysis between climate mitigation benefits and associated health risks.

4.12.P-Th180 Concentration Profiling and New Approach Methodology-Based Mixture Risk Assessment of Bisphenol A and Its Analogs in Foods

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The substitution of bisphenol A with structurally similar analogs has raised concerns due to their comparable endocrine-disrupting activities. Diet is considered the primary route of bisphenol (BP) exposure for the general population; however, limited data on exposure levels and toxicological information for BP analogs hinder comprehensive risk assessments. This study investigated 16 BPs in commonly consumed foods (n = 336) and assessed the mixture risk of endocrine activity resulting from co-exposure to multiple BPs in the Taiwanese population using new approach methodologies (NAMs). A representative food sampling list was designed using the core food method, and a UPLC–MS/MS analytical method coupled with QuEChERS extraction was developed and validated for determining BP concentrations in food samples. High-throughput toxicokinetic modeling was applied to convert in vitro ToxCast high-throughput screening endocrine bioactivity concentrations to human equivalent doses (HEDs), which were subsequently used to assess mixture risks through the margin of exposure (MOE) approach. The analytical method met regulatory validation criteria, with correlation coefficients of calibration curves > 0.99, recoveries within 60–125%, and relative percent differences < 30%. Eleven BPs were detected, with BPA showing the highest detection frequency (61.0%), followed by BPS (25.3%) and 4,4-BPF (20.8%). The mean concentrations of the sum of the 11 detected BPs (Σ11BPs) in foods ranged from 0.06 to 33.09 ng/g. The risk assessment indicated a low concern for endocrine activation from dietary co-exposure to BPs, though a moderate concern was identified from a more conservative perspective. This study enhances understanding of the dietary risks from BP mixtures and demonstrates the utility of NAMs in assessing human health risks posed by environmental contaminants.

4.13 Plastics in Terrestrial Environments: Connecting Uses, Fate, Exposure, Impacts and Innovations on Soil Health

4.13.T-01 Field Relevant Microplastic Concentrations Affect Soil Ecosystem Functioning in Mesocosms

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The increasing use of plastics in agriculture, for instance mulching film, contributes to plastic residues in soils. Soil microplastic (MP) concentrations are expected to keep rising due to growing use of agricultural plastics, the application of plastic-contaminated biosolids and aerial deposition of MPs. Experiments to date revealed initial effects of MPs on soil physicochemical properties, invertebrates, microorganisms and plants, but rarely studied complex interactions between organisms in the soil. Therefore, this study evaluated the effects of MPs on soil ecosystem functioning using a more holistic approach that reflects these complex ecological interactions using environmentally realistic mesocosm systems. A simulated agricultural ecosystem was made using Lufa 2.2. natural soil in which we introduced two species of earthworms (*Aporrectodea caliginosa* and *Dendrobaena veneta*), three species of springtails (*Heteromurus nitidus*, *Ceratophysella denticulata* and *Protaphorura fimata*) and lettuce (*Lactuca sativa*) as crop. MP types tested were conventional low-density polyethylene (LDPE) and biodegradable starch-polybutadiene adipate terephthalate (PBAT) blend, both sourced from agricultural mulching films. The aim was to investigate the interactions and impacts on physical, (bio)chemical and biomolecular responses within the system after 13 weeks of exposure. Soil physicochemical properties were affected by both MP types. Springtail community composition was not affected, nor was earthworm survival and reproduction, although molecular biomarkers show affected Electron Transfer System and Glutathione S-transferase activity in the earthworms, indicating oxidative stress. Plants exhibited adverse effects, showing strong activation of defense mechanisms and lowered chlorophyll content. Microbial community composition changed due to MP exposure and microbial activity was significantly enhanced. By combining all non-correlating and non-redundant response variables into a single multivariate analysis, we show that the overall profile of soil properties and biological functioning of the soil system significantly shifted in a treatment-specific manner, already at environmentally relevant concentrations of 0.05% LDPE (w/w dry soil). By linking MP-induced effects on soil properties, fauna and flora, this study demonstrates that mulching film-derived MPs, even at levels currently observed in the environment, may pose a risk to agricultural soil health and functioning.

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4.13.T-02 Biodegradable Microplastics in a Realistic Soil–Plant–Earthworms Set Up

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From the 1950s, plastics began to be widely used in agriculture with low-density polyethylene (LDPE) mulching. These films have revolutionized cropping by improving the soil microclimate and creating better growing conditions. However, once in contact with the soil, they gradually deteriorate and break down into microplastics. To reduce LDPE accumulation, biodegradable mulches were introduced in the 1980s as a sustainable alternative. They aim to be incorporated directly into the soil after use, where they micronise through weathering and biodegrade by soil organisms. Since both biodegradable mulches and LDPE mulches are a source of microplastics into agricultural soils, to gain understanding of their corresponding impacts in real cultivated crops, the present study assessed the effects of their microplastics on spinach plants and earthworms. Microplastics were produced from a commercial biodegradable mulch and LDPE pellets, and incorporated into pots filled with a substrate at 0.25% and 1% (w/w) and incubated for 5 weeks in a greenhouse. After incubation, earthworms (*A. caliginosa* and *A. chlorotica*) and/or spinach plantlets were added to the corresponding treatments resulting in 20 experimental conditions, including controls. At harvest, substrate samples were analysed for total organic carbon, total nitrogen, and weights. Spinach plants were assessed for weights, photosynthetic pigments, tannins, phenolics, and malondialdehyde. Earthworms were evaluated for fresh weight and malondialdehyde.

Microplastics from biodegradable mulch or LDPE pellets did not affect the substrate parameters. Spinach plant

weight was not affected by the presence of microplastics nor earthworms, though low biodegradable mulch microplastics concentration tended to increase chlorophyll a and b while high doses tended to decrease them. Carotenoids were enhanced by the high dose of LDPE. Lipid peroxidation was inhibited by the presence of microplastics and earthworms. Overall, the presence of earthworms buffered the effects of microplastics on spinach. Malondialdehyde remained mainly unchanged for the two earthworm species for all treatments. These results may indicate the activation of antioxidants defence systems, preventing strong lipid peroxidation. Microplastics contamination had limited effects on the substrate, or on spinach and earthworms physiology. These results highlight the key role of earthworms in buffering the biological effects of microplastics in soil-plant systems.

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4.13.T-03 Polymer- and Size-Specific Effects of Microplastics on Soil Respiration and Implications for Phytoremediation

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Soils are major sinks for microplastics (MPs), where synthetic polymers can persist for decades and alter soil structure, biogeochemical parameters and microbial activity. Soil respiration (SR), the combined CO₂ efflux from microbial decomposition and root respiration, is a key integrative indicator of soil functioning and carbon cycling, and thus a useful indicator to assess MP impacts. Phytoremediation (PR) nature-based option with fast-growing woody species such as *Salix caprea* could be used to restore soil functioning and health. Links between MP properties, SR responses and potential of PR to restore negative MP impacts remain poorly investigated. Here, we investigated how MP polymer type and particle size affect SR in two different soil types, and whether *S. caprea* can contribute to MP mitigation. MPs of polypropylene (PP) and polyamide 6 (PA) were produced in three size classes (1–3, 63–100, 100–200 µm) and added (1 wt%) to a sandy forest soil and a silty loam agricultural soil. SR was monitored over 28 days in an automated incubation system. In parallel, pot experiments with *S. caprea* were conducted in sand spiked with MPs of low-density polyethylene (LDPE) and PA in small (1–30 µm) and larger (2400–1200 µm) size fractions. Plant growth, root-MP interactions and MP recovery were assessed. MPs affected SR within 28 days in a size-, polymer- and soil-dependent manner. Larger particles generally increased SR, whereas the smallest size classes tended to reduce SR, particularly for PP in the loamy agricultural soil. We attribute these trends to structural effects on pore space and oxygen transport combined with polymer-specific bioavailability. More polar, water-absorbing PA consistently stimulated SR more than PP. In the PR experiment, *S. caprea* tolerated high MP loads without growth impairment and immobilised larger MPs on roots via fine root hairs, but overall MP removal from the substrate was low and very small particles remained in the pots. Our findings show that MP size and chemical properties modulate SR and thus soil carbon cycling. PR with *S. caprea* was mainly effective via root immobilisation of larger MPs. This work highlights SR could be a useful parameter to monitor the effect of MP remediation technologies on soil health and carbon cycling. Nature-based mitigation measures show a high potential but a better understanding of fate and root uptake of small MPs and effects on plants is needed in future work.

4.13.T-04 Freeze-thaw cycles can accelerate the vertical migration of pesticides and microplastics in soil.

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Keywords: Microplastic, Pesticide, Freeze-thaw, Vertical migration, soil column

Microplastics (MPs) are pervasive soil contaminants that can modify soil structure, influence water flow, and alter the fate of co-occurring agrochemicals through their hydrophobic surfaces and strong sorption interactions. Freeze-thaw (FT) cycles, common in temperate agricultural regions, further fragment plastics, increase soil porosity, and accelerate chemical release, collectively influencing pollutant transport. Here, we systematically evaluated how polyethylene (PE) MPs, FT cycles/room temperature, and soil type (sandy reference soil and clay field soil) regulate the vertical migration of twenty pesticides with diverse physicochemical properties using controlled soil-column experiments. Breakthrough curves, 5% and 50% breakthrough volumes (BVs), and

microplastic migration were quantified. FT cycles consistently enhanced pesticide leaching, with sandy reference soil groups exhibiting the highest leaching rate, whereas room-temperature field soils retained pesticides most effectively. Correlation analysis identified log KOC as a dominant predictor of migration behavior, while water solubility influenced leaching only in organic-rich soils. FT cycles also increased the downward transport of PE MPs. This study highlights how soil texture, climatic stressors, and compound properties jointly control pesticide mobility and microplastic redistribution, with implications for groundwater contamination risk.

4.13.T-05 Microplastic Risks in Soils Are Fragmentation Kinetics– and thus Time-Dependent

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Ongoing plastic emission and plastic fragmentation that increase particles' bioaccessibility over time call for a prospective ecological risk assessment framework. Thus, we developed a framework that incorporates a temporal dimension to fragmenting microplastics in soils, capturing their time-dependent behaviour and ecological risks. Two types of polymer coated fertilizers (PCF), an agriculture product with growing demands, were used as a case study.

The fragmentation model with the fragmentation rate optimized to fit measured PCF concentration data of a long term field experiment with yearly PCF application (sampled after seven years) was well able to describe the data. Fragmentation rate, particle's number, size and volume over time were calculated using the calibrated model. Food-dilution and physical- blocking-based effect thresholds, both of which are dependent on particle volume and size, were used to construct species sensitivity distributions (SSDs) and to estimate hazardous concentrations affecting 5% of species (HC5). Exposure and effects data were aligned for consistent risk characterization. Simulations were run to predict the particles' fragmentation and risks for the two PCFs for the time period of 30 years for both single and multiple application of PCF. The fragmentation model fitted well to both measured PCF concentration data and resulted in different fragmentation rates. As fragmentation progresses, SSDs, HC5 values, and corresponding risk characterization ratios shift, reflecting the increasing bioavailability and potential risk of smaller PCF-derived particles. Repeated PCF applications are predicted to elevate ecological risk, with the magnitude of risk significantly influenced by both the fragmentation rates and reapplication frequency. Besides community ecological risk, species- specific risks were also calculated to detect potentially endangered species over the investigated time period. Calculation was performed probabilistically to capture uncertainty in fragmentation kinetics and data alignments.

The proposed framework provides a dynamic, time-dependent ecological risk assessment for fragmenting microplastics. Its application may advance current risk assessment practices for microplastics through explicitly linking and aligning microplastic fragmentation kinetics to exposure and effects, supporting regulatory evaluation of PCFs, as well as other fragmenting microplastics products.

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4.13.P Plastics in Terrestrial Environments: Connecting Uses, Fate, Exposure, Impacts and Innovations on Soil Health

4.13.P-Mo303 Biodegradation of 14C-radiolabelled Natural Polymers in Soil in the Presence of Active Coal

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The biodegradability of polymers is determined according to OECD test guidelines (TG), for example TG 301 and TG 307. As synthetic polymers are restricted due to the EU Regulation (2023/2055) producers strive to shift to natural polymers, which are considered biodegradable by default, and synthetic polymers which show proof of fast biodegradation (i.e. >60% mineralisation measured in TG 301). The degradation rate and formed degradation products of respective polymers are often unknown. Even though natural polymers are considered

biodegradable this does not mean that they would pass those degradation tests. In our project an OECD TG 301 test showed that combining different natural polymers in a seed coating resulted in less than 60% mineralisation in 28 d. Furthermore, the presence of active coal in the seed coating has shown a decreasing effect on the biodegradability. Therefore, a simulation test (OECD TG 307) was chosen to learn more about the degradation behaviour of this seed coating material. For this purpose, the seed coating was produced with ¹⁴C-radiolabelled cellulose, sodium alginate and active coal as well as without active coal. The ¹⁴C-radiolabel allows to follow degradation processes in the complex soil matrix and to determine mineralisation (CO₂ formation) even at environmentally relevant polymer concentrations.

In the two tested soil types the difference between the mineralisation of the seed coating with and without active coal can be observed. Overall, it shows higher ¹⁴CO₂ formation in a loamy soil than a sandy soil. A water extraction of mono- and oligosaccharides is followed by an EDTA extraction of soil samples taken at different time intervals. The extraction was developed to quantify the degradation of the alginate (pyrolysis-gas chromatography and mass spectrometry (GC/MS)) as well as measure a shift in the molar mass (field-flow fractionation coupled to a multi-angle light scattering detector (FFF-MALS)). The amount of mono- or oligosaccharides extracted with water did not change substantially between 0 d and 28 d for both soil types, which could be due to mono- and oligosaccharides being degraded to ¹⁴CO₂ while the polymer chains are also degraded to mono- and oligosaccharides. The EDTA extraction has shown a recovery of the alginate from the seed coating of 70% with FFF-MALS-RI. The incubation time of the OECD TG 307 is finished in December 2025 and will be presented with the finalized extraction results at SETAC 2026.

4.13.P-Mo304 The Fate of Composted Compostable Plastics

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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 additives and non-intentionally added substances (so-called NIAS) - which can 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 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 characterized and quantified the biodegradable plastic fragments in the compost from a Dutch municipal composting plant. The characterized compost was placed in mesocosms on a Dutch field and sampled at the beginning of the experiment and after 3, 6 and 12 months (18 and 24 months are yet to be sampled). These samples are analyzed via NMR (Nuclear Magnetic Resonance) spectroscopy for a mass-based monitoring, allowing comparisons of mass and particle reduction.

Further we will try to assess the chemical composition of compostable biowaste collection bags and how the chemical leachate from compostable plastic products affect earthworm fitness, reproduction and development.

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 bags 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.

Disclaimer/Disclosure:

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4.13.P-Mo305 The soil legacy of biodegradable and conventional plastic mulching films: impacts on plant performance and soil invertebrate

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There is limited knowledge on the terrestrial hazards of mulching films and their potential microplastic release after field exposure. To address this gap, a research was carried out to investigate the legacy effects of mulching film application on soil. Two mulching films, one polyethylene (PE) and one poly(butylene adipate-co-terephthalate) (PBAT), were tested. The films were buried 10 cm deep below the soil under a Mediterranean climate. Soil samples containing microplastics released from PE and PBAT (coded as PE-soil and PBAT-soil, respectively) were collected after 32 and 276 days. After the exposure, we tested the effects of soils on terrestrial crustacean woodlouse *Porcellio scaber* (2 weeks) and two crops: the sunflower *Helianthus annuus* and maize *Zea mays* (8 weeks). Soil properties (pH, water holding capacity), woodlouse immune and detoxification responses, and plant physiological traits were assessed. Microplastics released from mulching films did not alter soil water holding capacity, but PBAT-soil collected after 276 days had a lower pH. *P. scaber* showed no significant changes in enzyme activities or immune parameters. Also, most plant physiological traits (transpiration rates, potential photochemical efficiency (Fv/Fm), actual photochemical efficiency (Y) and chlorophyll a/b ratio remained unaffected in sunflower and corn. However, some changes were observed in plants. Sunflower shoots grew better in PE-soil collected after 32 days (higher shoot fresh weight), but not in the case of soil collected after 276 days. After this latter period, PBAT-soil increased sunflower root water content, but reduced root dry weight and PE-soil induced oxidative stress in sunflower roots. In corn, elevated root proline at both time points and lower root water content was found when cultured in PBAT-soil collected after 276 days. We conclude that mulching films in general did not induce severe effects on soil, but some changes were induced by both mulching films irrespective of their biodegradability potential.

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4.13.P-Mo306 From Soil Contaminant to Drought-Like Stress Inducer: Physiological and Biochemical Responses of Sunflower to Microplastics from Biodegradable Bags

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Biodegradable plastics are promoted as sustainable alternatives to conventional polymers, yet fragmentation into microplastics (MPs) introduces new stressors to terrestrial systems. MPs can alter soil physicochemical properties and plant physiology, but whether they induce drought-like stress compared with conventional polymers remains unclear. We tested if pristine MPs from two poly(butylene adipate-co-terephthalate) bags suitable for home (PBAT_HOME) and industrial (PBAT_IND) composting trigger drought-like responses in sunflower (*Helianthus annuus* L.), compared with MPs from conventional polyethylene (PE) bags. Seedlings were grown in soil spiked with MPs at 0, 0.05%, 0.5% and 1% w/w. Polyethylene glycol (PEG) at the same concentrations was included as a positive control treatment for drought-like responses, because it is a well-established early biomarker of drought and osmotic stress. We quantified growth (fresh and dry weight of shoots and roots), photosynthetic pigments (chlorophylls, carotenoids), actual and potential photosynthetic efficiency, biochemical markers (H₂O₂, proline, total soluble sugars), as well as soil water holding capacity and pH.

PE had minimal effects on plant growth at all endpoints. In contrast, PBAT MPs caused dose-dependent stress responses, strongest at 1%: shoot and root biomass decreased, total chlorophyll content declined with a relative increase in carotenoids. Proline increased in shoots, while H₂O₂ and total soluble sugars increased in both roots and shoots, consistent with oxidative stress. Two-way clustering analysis of physiological and biochemical variables grouped both 1% PBAT treatments with all three PEG treatments, indicating a classical water-deficit signature rather than a nonspecific toxicity pattern. Soil responses were polymer-specific: WHC increased by 1% for all three types of MP, while pH increased only for 1% PBAT_IND compared to the control samples, indicating that different chemical properties of polymers can alter soil properties in different ways, affecting the plant water status.

Overall, our results show that pristine PBAT MPs—despite being labelled "biodegradable"—can cause drought-like stress in one of the major crops, while PE MPs have minimal impact. These findings challenge the

assumption that compostable plastics are agronomically harmless and highlight the need for polymer-specific risk assessment frameworks that integrate plant physiology with soil property changes.

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4.13.P-Mo307 Impacts of Low-Density Polyethylene (LDPE) microplastics (MPs) on soil organism growth and functioning: influence of additive dose

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Plastics, such as the most widely produced and used plastic, low-density polyethylene (LDPE), are made up of a main polymer and a large group of substances known as 'additives'. MPs additives are most likely to enter the soil environment. Additives are then suspected to play a role in the toxic effects generated by MPs, but the effects of these leachates have not yet been thoroughly assessed and all current scientific data focus on additive characterisation in soil or on additive effects on a single organism.

The aim of the work presented was to answer the following question: what are the effects of MPs and what is the influence of the additive dose on the growth of soil organisms and soil functions at relevant environmental exposures?

The answer to this question was based on three hypotheses:

- 1) MPs have negative effects on the growth of organisms
- 2) The concentration of additives is involved in the toxicity of MPs to soil organisms.
- 3) MPs and additives interfere with soil functioning.

To answer these questions, we worked with three biological models: two endogeic annelids (*Aporrectodea caliginosa* and *Enchytraeus albidus*) and a monocotyledonous forage plant: *Lolium perenne* L. These organisms were exposed according to three biological modalities, without interaction (one species), with one interaction (two species) and with two interactions (three species). To assess the effects of MPs and additive doses, we compared the growth of the organisms between three treatments: control (non-contaminated) - conventional MP exposure and "over-concentrated in additive" MPs exposure.

Regarding organism growth, no effect of MPs or additive dose was observed, neither in enchytraeids, earthworms nor in plants. Regarding microbial activities, the Denitrification Enzyme Activity is the only activity that responded to MPs exposure without interaction. In fact, no effects were observed on Substrate Induced Respiration and Nitrification Enzyme Activity.

We also observed that MPs and additives had adverse effects on the growth of all organisms when exposed in interaction with other organisms. Based on our results, it is likely that the toxicity of MPs is related to the leaching of additives into the soil, which may affect soil functioning. This work also shows that working with environmentally relevant organisms in interaction can help to understand the effects of a contamination on soil ecosystem functioning.

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4.13.P-Mo308 Indirect trophic transfer of nanoplastics in soil ecosystems: A three-level exposure model with lettuce, snails, and springtails

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Micro- and nanoplastics present in soil environments can be absorbed by plants and may reach higher trophic levels via animals that consume these plants. Therefore, studies on trophic transfer of micro- and nanoplastics within soil ecosystems are necessary. To this end, we aimed to observe the indirect transfer of nanoplastics within a three-tiered trophic system in soil ecosystem using lettuce, snails, and springtails. Fluorescent

nanoplastics were absorbed from the soil into lettuce, and plastic fluorescence was detected on the snail feces and the surface of springtails ingesting the snail feces. The transferred plastics were found to affect oxidative stress indicators such as microorganisms within snail feces. Furthermore, it was found that springtails disperse nanoplastics throughout the medium as they move after ingesting the feces. These findings suggest that micro- and nanoplastics in soil can be transferred and cycled throughout the entire soil ecosystem as well as higher trophic levels, emphasizing the need for plastic pollution management in food resources.

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4.13.P-Mo309 Terrestrial Ecotoxicity Studies for Microplastic Particles – Influence of particle size, shape, polymer type and UV aging

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Growing interest in the effects of microplastic particles (MPs) on ecosystems has led to a surge in related research. While early studies focused on marine systems, attention has recently shifted towards terrestrial environments. Concerns about the reliability and relevance of MP ecotoxicological studies often stem from inconsistent reporting of particle properties and limited environmental representativeness (de Ruijter et al., 2020; Mehinto et al., 2022).

This work aimed to improve the hazard and risk assessment of MPs in terrestrial systems by identifying testing and reporting challenges and proposing solutions. Within the Holistic Environmental Risk Assessment for Microplastics in the Terrestrial Environment project (ECO 61), a review of terrestrial effect studies on engineered nanoparticles and micro- and nanoplastics (MNP) was completed. A comparative analysis was conducted using a strengths, weaknesses, opportunities, and threats (SWOT) framework.

A tiered testing approach was applied: pristine particles were studied in Tier 1 and UV-aged particles in Tier 2. In Tier 1, long-term effects of six MPs differing in shape, size, and polymer type were tested on soil microorganisms (OECD TG 216), plants (ISO 22030), and earthworms (OECD TG 222). Glass particles served as a negative control. Test systems were designed to minimize contamination and ensure compliance with quality assurance (QA) and quality control (QC) guidelines. Concentrations ranged from 0.1 to 1000 mg/kg dry weight soil to encompass environmentally relevant and worst-case exposures. In Tier 2, three of the particles were UV-aged following OECD TG 316 recommendations and retested.

QA/QC criteria were met across systems, with only minor deviations. Results suggest current regulatory test systems are sufficiently conservative to assess potential effects. Significant effects generally appeared at 100 and 1000 mg/kg soil. There was no clear indication that one of the organisms was most sensitive or that polymer type, size or shape are the main drivers for effects. However, UV aging appeared to enhance toxicity in some materials, particularly towards soil microorganisms. In plant tests, glass beads indicated a possible 20% background effect from particle presence alone.

These findings will be used to develop a holistic environmental risk assessment framework for MPs in terrestrial ecosystems now.

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4.13.P-Mo310 Assessing the ecotoxicity of polypropylene fragments on earthworm with soil covering and mixing exposure scenarios

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Even though microplastics would initially emit to surface of environmental media in the realistic soil environment, there are limited research on considering different exposure scenarios of microplastics to earthworms. The purpose of the present study is to evaluate the ecotoxicity of microplastic fragments on earthworms which an important ecological indicator for soil pollution. Polypropylene (PP) fragments from products and earthworm *Eisenia fetida* were selected as model microplastics and test species. To compare the different exposure scenarios, column soil test and whole mixed soil test were conducted. In the column soil test,

amended soil with PP fragments were placed on the topsoil. After 3 weeks, earthworms avoided amended soil with PP fragments and then mortality, reproduction inhibition, *in vivo* cytotoxicity were not observed in the column test. On the other hand, inhibitions in lysosomal stability of earthworm cells, male and female reproductive tissues were significantly observed when earthworms were exposed by PP fragments thoroughly during whole exposure period (3 weeks). These results may be associated with the reduction of exposure level (concentration and period) due to the avoidance behavioural reactions of earthworms to pollutants in the column test.

4.13.P-Mo311 (Eco)toxicological effects on humans and on biota from micro/nanoplastics in relation with agrochemical applications - combined exposure of nano-pesticides and plastics

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Nano- and microplastic occurrence is widely evidenced across several environments, marine, freshwater, and soil. This includes agricultural soils, and various studies investigate the occurrence of plastics in agricultural soils. An increasing number of scientific articles report for example the enhanced efficacy of nanoplastics when combined with chemicals like pesticides, and this co-exposure may have synergistic effects. The data of such studies with pesticide are of high importance as this evidence can serve in resolving several issues in risk assessments, like combined effects (with other pollutants) that occur under realistic conditions. However, we still know very little on the (eco) toxicological effects on humans and on biota from micro/nanoplastics in relation with agrochemical applications, and it is also not clear how this information could be utilized in the field of regulatory risk assessments. A systematic literature review was therefore conducted to investigate: (i) the (eco)toxicological effects from combined exposure to nano- pesticide and plastic, and if we need to focus on specific toxicological impacts; (ii) if and which of the impacts on the organisms of the environment (humans and biota) from these combined exposures to (nano) pesticide and plastic, are assessed or sufficiently investigated to serve for regulatory risk or effect assessment schemes; (iii) which are the advances in nanomaterials in relation to ecotoxicological effects from pesticide and (micro/nano)plastic - combined exposure? This search aimed to shed light on how to utilize this information in novel or alternative regulatory risk assessments, and thus to facilitate minimization of adverse effects on environmental and human health.

4.13.P-Mo312 Towards a Holistic Hazard Assessment Framework for Microplastics in Terrestrial Environments

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Microplastics (MPs) are increasingly recognised as pollutants of concern in terrestrial environments, yet current risk assessment approaches struggle to capture their particulate behaviour and environmental complexity. In this work, we outline a new hazard assessment framework for MPs in soil, developed as part of a wider research programme (ECO61, CEFIC-LRI). The framework takes inspiration from established chemical regulatory systems and adapts them to address the practical and conceptual gaps surrounding microplastic testing and evaluation.

We propose a tiered testing approach that brings together OECD and ISO methods to assess biological responses at multiple levels, from microbes and invertebrates to plants. Central to this framework is a call for more consistent particle characterisation, covering aspects such as shape, size, polymer type, surface charge, and ageing. Building on lessons learned from evaluating engineered nanomaterials (ENMs), the framework also stresses the need for test materials that better reflect environmental conditions, particularly fibrous and aged MPs, which are common in real soils but rarely included in standard laboratory tests.

Different methods for deriving Predicted No-Effect Concentrations (PNECs) are discussed, including traditional assessment factors, species sensitivity distributions (SSDs), and weight-of-evidence approaches. We also highlight ongoing challenges with data quality, reproducibility, and the lack of harmonised test standards. Furthermore, the framework makes recommendations for improving consistency, developing reference materials, and linking MP hazard assessment more closely with soil health indicators.

Overall, this work aims to help researchers and regulators move toward more realistic, evidence-based evaluations of microplastic hazards in terrestrial ecosystems.

4.13.P-Mo313 UV-Induced Microplastic Formation and Pollutant Release from Cigarette Butts: Insights from Soil Column Migration and TED-GC/MS Analysis

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Keywords: Microplastic, Cigarette butts, Soil column, Ultraviolet aging, Vertical migration

Cigarette butts (CBs) represent one of the most pervasive anthropogenic litter items worldwide, yet their transformation into microplastics and their role as sources of emerging pollutants in the terrestrial environment remains poorly understood. This study investigates the UV-induced aging of cigarette butts on soil cores, focusing on the formation of microplastics and the release of associated chemicals, as well as their subsequent migration behavior in soil. Controlled UV-aging experiments were conducted to simulate environmental weathering, followed by soil-column studies to evaluate the vertical transport of CB-derived microplastics and pollutants under real-world infiltration conditions. A microplastic detection strategy was established using Thermal Extraction-Desorption Gas Chromatography-Mass Spectrometry (TED-GC/MS), enabling high-resolution quantification of polymer residues along soil profiles. Complementarily, non-targeted analytical approaches were employed to identify and characterize aged-CB-derived organic chemicals released into soils. The findings address the rates and mechanisms of microplastic generation from cigarette butts, identify key pollutant species mobilized during UV aging, and elucidate their migration pathways in soil. This work provides crucial insight into CB-related environmental risks and supports the development of targeted strategies for pollution mitigation in terrestrial ecosystems.

4.13.P-Mo314 Azoles and other pesticides/biocides sorbed to plastic could pose a threat

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Azoles are a class of antifungal agents widely used in medicine, agriculture, and various industries. They play a critical role in treating fungal infections, which cause over two million deaths globally each year. However, their extensive use in agriculture, cosmetics, medicine, including veterinary medicine, wood protection, and even golf course maintenance has contributed to increasing fungal resistance, threatening both human and plant health. Recent research under the “Jekyll and Hyde” project, led by the Norwegian Veterinary Institute in collaboration with NIBIO and others, investigates whether plastic waste in nature can act as a carrier for harmful fungi and azoles. Volunteers across Norway collected plastic samples from diverse environments, including forests, fields, and mountains. From these, 120 representative samples were analyzed for microbial presence, azoles, and chemical composition.

Surprisingly, NIBIO detected pesticides and other pollutants in 25 plastic litter samples and azoles in 14 of the samples. These azoles included active substances from plant protection products, antiparasitic agents, and biocides commonly used in Norway. Industrial chemicals such as methylbenzotriazole and benzisothiazolinone were also found, though their role in resistance remains unclear.

The findings suggest azoles bind more strongly to plastic than previously assumed, allowing contaminants to persist and spread beyond agricultural areas into natural habitats. This raises concerns about environmental transport of antifungal agents and the potential acceleration of resistance development, posing risks to health and ecosystems.

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4.13.P-Mo315 Aging of Microplastics in Soils: Effects on Surface Chemistry and Interactions with Organic Contaminants

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Microplastics are frequently accumulated in agricultural lands, with concentrations ranging from few to tens of thousands of particles per kg of soil. Despite their extensive occurrence, little is known about their long-term fate and physicochemical transformations in soil, processes that subsequently influence their behavior as sorbents for organic contaminants. Thus, this study investigated the fate of Linear Low-Density Polyethylene

(LLDPE) microplastics during soil incubation and how these transformations affect the surface properties of LLDPE and interactions with organic contaminants. To that end, LLDPE mulch film that had undergone one year of environmental weathering was ground to 0.7-1 mm particles and incubated for 20 months in three loess soils. Particles were extracted after 6, 11, 15, and 20 months and used as sorbent for organic contaminants. ATR-FTIR analyses were performed to characterize surface chemical changes.

Across soils, LLDPE aging resulted in a pronounced increase in polar functional groups (particularly C-O bonds) during the first six months, likely due to eco-corona formation, while polymer backbone alterations remained minimal. Sorption affinity (K_d values) ranged from <0.1 to 1721 mL/g, with diphenhydramine, venlafaxine, 6PPD-quinone, aspartame, and oxcarbazepine exhibiting the highest affinities. Nearly half of the compounds (42%) exhibited relatively low K_d values (<5 mL/g), with only minor effects of soil type. Soil aging of the LLDPE particles had a limited influence on the sorption affinities, with 90% of contaminants exhibiting minor changes, with only five compounds displaying a K_d increase of >10 , mainly within the first six months. Desorption was largely reversible, with hysteresis observed only for four contaminants.

Our data show that the sorption affinities varied widely across contaminants; nevertheless, K_d values measured for LLDPE microplastics were often of the same order of magnitude as those of the same contaminants in the soils or lower. Consequently, under environmentally realistic conditions, the contribution of LLDPE microplastics to overall contaminants retention and soil affinities is expected to be limited, while compound-microplastic specific interactions cannot be fully excluded.

4.13.P-Mo316 Microplastic contamination in agricultural soils: uFTIR method assessment and European survey of 220 fields.

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Plastic use in agriculture has tremendously increased in the past decades resulting in plastic contamination in soils. Few studies evaluated the contamination in different agricultural systems but each studies used a different protocols making them difficult to compare, especially when considering the smaller sizes of plastic debris. One method that is generally use to quantify microplastic contamination is density separation followed by imaging with FTIR spectroscopy. Within the MINAGRIS project, we worked with two laboratories (from the University of Bern and University of Wageningen) to establish and evaluate a microplastic quantification method with density separation and FTIR spectroscopy. Here we present first, the results from our quality controls from which we propose guidelines for microplastic assessment in soils, covering:

- Sample contamination using blank chemicals and blank soils
- Measurement variability across different operators, extractions and laboratories.
- Recoveries for three polymers in different spiked soils : Polyethylene (PE), Polylactic Acid (PLA) and Polyvinyl chloride (PVC)

Then we present the results for more than 220 agricultural fields across different pedoclimatic regions and agricultural systems in Europe. The soil was collected by teams in 11 different study sites, from 10 different countries. Our goal is to provide a snapshot of the microplastic contamination in Europe in 2022 and relate it to the different land managements.

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4.13.P-Mo317 Searching for Microplastics in Complex Matrices: Insights from an Interlaboratory Comparison

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Global plastic production continues to rise due to its versatility, durability, and use in modern society, leading to an increasing amount of macro- and micro- plastic materials entering the environment. These materials have a wide range of physicochemical properties, and the lack of harmonized procedures for sampling, matrix removal, and analysis has made cross-study comparisons difficult. Standardized methodologies are urgently needed to improve the reliability and comparability of microplastic measurements across environmental matrices.

In this work, we address the current absence of an agreed-upon extraction protocol for microplastics by

developing a standardized method for extraction of microplastics from compost, with a targeted publication as an ISO standard by February 2027. The method comprises deagglomeration, organic matter removal, and density separation, with each procedural step optimized through multiple iterations of method refinement.

We present the results of an interlaboratory comparison conducted to evaluate the performance of the proposed protocol. Nine laboratories participated, applying different analytical techniques, including micro-Raman, micro-FTIR, and Py-GC/MS, to quantify microplastics in shared test materials. All laboratories successfully reproduced the extraction process within their replicates, although interlaboratory variability was observed. The results and the feedback received enabled the identification of steps requiring further optimization. The comparison confirmed that LDPE and PBAT can be repeatedly and reproducibly extracted, and it is reasonable to assume that other low-density microplastics ($<1.4 \text{ g/cm}^3$) could be recovered using the same approach, provided they remain stable under Fenton treatment.

This emerging standard is intended to provide a robust foundation for generating more comparable microplastic data across laboratories and for advancing our understanding of microplastic occurrence in compost and similarly complex environmental matrices.

4.13.P-Mo318 Modelling the Transport of Microplastics Across Reactive Barriers in Aquifer Recharge System: A Case Study in Palamós

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Managed aquifer recharge (MAR) with reactive barriers (RBs) is a promising strategy to limit microplastic (MP) transfer from wastewater effluents to groundwater, but field-scale transport processes and retention mechanisms remain poorly constrained. We investigated MP behaviour in a pilot MAR system in Palamós (Spain) fed by secondary effluent from a municipal wastewater treatment plant. We focused on three infiltration lines: a sand reference (T2) and two organic-rich RBs, T4 (sand–compost–clay) and T5 (sand–woodchips–clay). MPs were quantified in water at the MAR inlet (INF), immediately below the barrier (OB) and at the aquifer outlet (C), and in barrier material sampled at two depths (L1, L3) after system dismantling. MPs were extracted by oxidative digestion and density separation, classified by morphology and size by stereomicroscopy, and identified by FTIR imaging.

We developed a one-dimensional advection–dispersion model including two solid-phase compartments for reversible and irreversible retention, with first-order attachment/detachment kinetics and a blocking/straining function. RB and aquifer segments were represented explicitly, using field-based hydraulic parameters and residence times. Model calibration targeted water-phase MPs/L at INF, OB and C and retained MPs/kg in barrier material.

MP concentrations decreased along the MAR system with strong contrasts among lines: total removal (INF?C) reached 33% (T2), 88% (T4) and 100% (T5). Organic RBs accumulated substantially more MPs, with higher loads near the barrier surface, indicating efficient near-inlet interception. Size distributions narrowed along the flow path and polymer diversity decreased, showing strong size- and polymer-selective filtration. The dual-site model successfully reproduced both water and solid-phase data, yielding higher attachment rates and greater irreversible retention in organic RBs than in the aquifer.

Our results demonstrate that organic-rich RBs embedded in MAR can significantly reduce MP fluxes to groundwater and that field-calibrated dual-site models provide a powerful tool to support design and upscaling of MP-mitigating MAR systems.

4.13.P-Mo319 Effects of Microplastic Polymer Types on Soil Hydraulic Response and Microplastic Transport During Rainfall Events

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Microplastics (MPs, diameter $< 5\text{mm}$) are widely detected in terrestrial environments, yet their fate in soil and their mobility during precipitation events remain insufficiently understood. In particular, it is unclear how different MP polymers influence soil structure and soil hydrological properties and, consequently, runoff patterns and MP transport. This study aims to determine how pristine LDPE, weathered LDPE, and weathered PBAT affect soil hydraulic properties, runoff characteristics, and sediment and plastic transport.

Loess soil flumes ($1 \text{ m} \times 0.5 \text{ m} \times 0.3 \text{ m}$) were individually spiked with 0.1% w/w MPs (pristine LDPE,

weathered LDPE, or weathered PBAT) and incubated under repeated wet–dry cycles for six months to promote soil aggregation. Following this incubation, the flumes were subjected to simulated rainfall at an intensity of 70–73 mm h⁻¹ for 30 minutes. Runoff was collected every three minutes until flow ceased. Ongoing analyses include soil properties such as water retention curves, saturated hydraulic conductivity, measured before and after the rainfall event, as well as MP partitioning across soil aggregate size fractions.

We hypothesize that soils containing LDPE will behave differently from those containing PBAT because LDPE's hydrophobicity and low density are expected to reduce water retention and decrease K_{sat}, which will subsequently lower soil infiltration, and accelerate runoff initiation, thereby enhancing the transport of LDPE particles due to their buoyancy. Furthermore, we expect pristine LDPE to exert a stronger influence than weathered LDPE, as its smoother surface and higher hydrophobicity. In contrast, weathered PBAT—being more hydrophilic—is expected to increase soil water-holding capacity, delay surface saturation, and produce later runoff initiation with lower sediment concentrations and reduced sediment/runoff ratios, while also resulting in comparatively lower PBAT transport due to its higher density and affinity for soil particles. Currently, soil property analysis and MP quantification are ongoing; at SETAC we expect to be able to present the results. This research advances understanding of MP–soil interactions under realistic rainfall conditions and provides new insight into the mechanisms controlling runoff and sediment transport as well as MP mobility in agricultural landscapes. The findings will help clarify whether soil will act as a sink or a source of MPs in the future.

4.14.P Soil Monitoring and Resilience - Which Chemicals, What Effects and How to Protect Ecosystem Functioning?

4.14.P-Th181 Prioritisation of contaminants for soil monitoring in Europe

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1. Introduction

The European Soil Monitoring Law (SML) introduces a need for systematic prioritization of contaminants for soil monitoring to address diffuse pollution. With thousands of chemicals regulated by different frameworks, prioritization must balance scientific robustness, regulatory coherence, data availability, and practical feasibility. This paper contributes to developing prioritization for the SML by comparing methodologies applied to soil contaminants from various frameworks, focusing on conceptual design, parameter selection, and handling data gaps. The work results from collaboration across EU Soil Mission and Horizon Europe projects in the Soil Pollution and Remediation cluster.

2. Materials and Methods

Several prioritization methods have emerged, addressing the environment and human health or single compartments like water. We analyse four approaches for soil pollutants:

- (i) risk-based scoring integrating persistence, mobility, bioaccumulation, ecotoxicology, and toxicity
- (ii) tiered frameworks combining emission models with measured soil data
- (iii) multi-criteria decision analysis (MCDA) aggregating hazard, exposure, and socio-economic indicators
- (iv) category-based schemes classifying substances into “action categories” before ranking.

A common dataset of 20 substances was tested with these approaches developed in Soil Mission projects (ARAGORN, ISLANDR), the Norman Network, and the German Environment Agency (UBA) on pesticides (data-rich), PFAS (data-poor), metals, and emerging substances to illustrate how methodological choices influence outcomes.

3. Results and Discussion

Key differences include reliance on occurrence versus proxy data, treatment of uncertainty, and capacity to integrate mixture effects or climate stressors. Simple scoring offers transparency and ease but may oversimplify risks. Risk-based, tiered, and MCDA approaches provide flexibility and accommodate diverse data contexts yet require harmonized input, which may not be publicly available. Category-based approaches help manage knowledge gaps and guide research priorities but depend on expert judgment.

4. Conclusions

Hybrid strategies combining risk-based logic with uncertainty indicators and adaptive mechanisms best address monitoring needs. Recommendations include harmonizing parameter definitions, improving data interoperability, and embedding prioritization tools into EU-wide and national programs to strengthen comparability and policy relevance.

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4.14.P-Th182 PFAS Contamination in Swiss Soils – an Overview

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Due to their persistence, toxicity, and their widespread use, per- and polyfluoroalkyl substances (PFASs) are a reason for concern for the environment and human health. Therefore, threshold values for chemical soil protection are needed. In order to set these values and prioritize measures, knowledge on PFAS concentrations in Swiss soils is crucial.

Information on background concentrations of PFASs in Swiss soil was published by Thalmann et al. in 2022. However, further knowledge on soil contamination is limited. To improve this, a data collection of measurements of PFASs in Swiss soils was initiated. Measurement results from over 1000 samples were gathered with most of the data originating from cantonal measurement campaigns. The objectives of these campaigns varied, some assessed background concentrations of PFASs in soil, while others screened for potentially contaminated sites or assessed known contaminated sites further.

This study summarizes the current knowledge on PFAS soil levels in Switzerland. It sheds light on which PFASs are found at the highest concentrations and which PFASs have the highest detection frequencies. Furthermore, it identifies relevant PFAS sources and concentration differences between land-uses. Particular attention was paid to understand substance patterns associated with different PFAS applications or land-uses. We were able to show that the application of sewage sludge, the use of firefighting foam as well as diffuse pollution are associated with specific substance-patterns. An in-depth cluster-analysis was performed to determine whether other parameters (such as location, suspected PFAS-sources or soil characteristics) can explain differences in the frequency of substance detection and measured concentrations.

These results improve our understanding of the concentration of PFASs in soil and contribute to the derivation of soil threshold values in Switzerland.

4.14.P-Th183 Normal operating ranges in soil ecotoxicology: the development of effect-based thresholds to assess the impact of chemicals on soils

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In ecotoxicology, the biological response of organisms in reference conditions is necessary to interpret responses obtained in contaminated conditions. In the case of retrospective assessments of diffuse pollution in soils, it is not always possible to obtain a representative uncontaminated natural reference soil. Furthermore, artificial soils used in standardized bioassays (e.g. OECD soil) are not always representative of the soil properties in the study site. As soil organisms are sensitive to soil properties, when comparing different soils it can sometimes be difficult to determine how much of the response observed is due to contamination or to differences in soil properties.

One way to address this issue is to characterize the natural variability of the biological responses (e.g. survival,

growth, reproduction) obtained on different natural reference soils with different geochemical properties. Defining a Normal Operating Range (NOR) for several soil bioindicators would provide a reference based on the effect of soil properties on the variation of the biological response. The normal response thresholds can then allow evaluating if the effects of suspected contaminated sites fall outside the natural variability, in cases where establishing reference sites is challenging.

To demonstrate this concept, a pilot study was conducted with agricultural soils (temporary prairies or organically managed) from the Jura region of Switzerland. The biological responses on these soils were assessed using 4 species: *Folsomia candida* (ISO 11267), *Enchytraeus crypticus* (ISO 15387), *Allium cepa* and *Brassica napus* (ISO 18763). To establish these NOR, two statistical approaches were compared: one assuming a normal distribution and calculating percentiles (2.5 and 97.5) as thresholds. The second based on the Minimum Detectable Difference (MDD) expressing the inherent variability, and the Maximum Detectable Difference (MTI) representing the variability between the reference soils in comparison to a control soil (e.g. LUFA).

In general, it was possible to develop normal operating ranges for all bioassays. However, the approach based on MDD/MTI which is dependent on a control response (LUFA 2.2) is less adequate if the control soil is not representative of the region. The current study is an initial pilot and must now be extended to other biogeographic regions of Switzerland, but these tools are a promising approach to be used in monitoring contexts.

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4.14.P-Th184 A novel Mixture Risk Assessment Approach for Agricultural Soils put to the Test: Which Trophic Levels are at Risk in Soil Field Samples?

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Plant protection products (PPP) are applied in agricultural fields for crop protection and may accumulate in the soil. As a result from serial applications or slow degradations of PPP, the soil and organisms therein may be exposed to a mixture of PPP residues. However, single substance environmental risk assessment in soil does not consider the added risk, which may arise due to co-exposure with other substances. We here present a novel mixture risk assessment approach for soils, termed RQtaxa, which assesses four trophic levels in soils for their potential risk due to exposure to multiple PPP residues. The RQtaxa approach has the advantage that it does not exceed the conventional method of summing up all risk quotients of detected PPP residues in a given mixture but differentiates by organism groups. The RQtaxa concept was applied to soil field samples from the Swiss national soil monitoring network, which also contained information on the crop type of the soil samples. This exercise provides insights into which soil organism groups are at risk in the various crop types and reveals data gaps on missing soil organism toxicity data, which helps to steer further ecotoxicity testing. In addition, it also highlights how many PPP residues drive the mixture risk in which crop types. With the recently endorsed EU soil monitoring law, the RQtaxa concept would be an interesting option to consider mixture risk assessment on an international scale.

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4.14.P-Th185 Soil Risk Assessment of Pesticides: Issues with Using Regulatory and Research Data in the Retrospective Risk Assessment

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In order to maintain health and function, agricultural soils need to be regularly evaluated: pesticide residues are monitored and used in conjunction with soil ecotoxicity data in a retrospective risk assessment. For this purpose,

we evaluated regulatory and research studies on pesticides using the modified, soil-specific Criteria for Reporting and Evaluating Ecotoxicity Data (CRED) with some amendments (e.g. exposure must be through soil, no recovery accepted). We faced two major groups of issues: the quality of the studies and their reporting. Regarding the study quality, common issues are lacking analytical verification, unknown test organism sensitivity (positive control), missing solvent control and incomplete statistical evaluation. Regulatory study reports are still rarely available publicly, usually only the study summaries in regulatory dossiers. These summaries often omit important details, such as soil properties or specifics of the test item application. In research articles the test item is often not properly identified, pesticide use history of the sampled/test field is lacking or field collected test animals are used. General issues in presenting the outcome include not reporting the data for the effect results (at least mean value $\pm$ SD per treatment for all measured effects), for the statistical results (e.g. statistical significance, fitted curve, goodness of fit, confidence intervals) or for the validity criteria as defined by standard test guidelines.

These issues resulted in a large number of refusals of potentially relevant studies. As a consequence, the derived risk-based hazard values are less robust and in case of resulting data gaps higher assessment factors need to be selected.

In addition, we observed that the results of the seedling emergence test on terrestrial plants following the standard test method are difficult to compare with the results on other soil organisms (application rate instead of concentration in soil is used), while the test with predatory mite seems to be superfluous based on its apparent relative insensitivity to pesticide exposure.

Regarding the regulatory studies, some points identified above might be addressed in the ongoing revision of the SANCO guidance document on terrestrial ecotoxicology. The here applied soil-specific CRED guideline is highly recommended to be used in order to minimise the gap between research and regulatory frameworks.

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4.14.P-Th186 Effects Of Reduced Application of Pesticides On Soil Residue Concentrations And Soil Invertebrates

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Pesticides are widely used in conventional agriculture to protect crops and to support yields, but once applied, they can reach the soil where many persist for years, resulting in the chronic exposure of soil organisms. Soil biota play several functions that are crucial for maintaining soil health. There is increasing evidence that pesticides have an adverse effect on the fitness of soil organisms, which can result in shifts in communities and ultimately in changes in soil functions. A more sustainable approach to agriculture to minimize the impact on soil organisms is needed. The PestiRed project aims to reduce the pesticide use by 75% applying alternative measures for weed, pest and disease control, based mostly on agroecological principles. A maximum yield loss of 10% is allowed. Such innovative measures are tested on several farms across three Swiss regions. On each farm, one plot is managed conventionally while innovative measures and pesticides reduction are applied to a second plot.

To investigate the consequences of pesticide reduction on soil contamination, concentrations of 146 residues are measured in both plot types of the PestiRed farms. Measured environmental concentrations are then compared with application data, as well as with ecotoxicological data to assess potential risk to soil biota. In a second step, the same samples are used to evaluate the fitness of two model invertebrates after exposure to the real soil residue concentrations.

Preliminary results suggest that residue concentrations decreased with time and management change, but this reduction remained modest and did not fully reflect the 75% reduction in the application. Site-specific characteristics and substance properties likely contribute to this discrepancy. Similarly, ecotoxicological assays showed large variability in the reproduction response, suggesting that additional factors, beyond management alone influence the results. More in-depth analysis will be used to further explore the contribution of the different drivers.

Combining complementary indicators of soil health (chemical, ecotoxicological, ecological) provides a more comprehensive approach to addressing the problematic from different lines of evidence, enabling an integrated assessment of the management effect on soil health.

4.14.P-Th187 Effects of Fluroxypyr on Soil Mesofauna Assessed by Non-Parametric Indicators in Field Mesocosms

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Fluroxypyr is a widely used auxinic herbicide, yet its potential to disturb soil mesofauna remains poorly understood, despite recent laboratory evidence indicating toxicity to non-target invertebrates. This knowledge gap is especially relevant for belowground ecosystems, where pesticide impacts are often inferred rather than measured under realistic conditions. The present study aimed to assess the sensitivity of soil mesofauna to fluroxypyr using non-parametric indicators in a controlled field mesocosm experiment.

Fluroxypyr, applied as a commercial plant protection product, was tested in a field mesocosm experiment using intact soil cores exposed to five agriculturally relevant application rates (0–400 g a.i. ha⁻¹) and incubated under natural conditions for 20 days. Soil fauna were extracted and identified at the family level. Soil biological quality was assessed using a modified, abundance-weighted QBS-Ar index, while community-level responses were evaluated based on selected microarthropod taxa and soil microbial functioning was quantified by measuring soil respiration.

Fluroxypyr application significantly affected selected soil organisms and functional indicators. Among Collembola, only family Isotomidae showed a significant response to treatment, with abundance reduced relative to the control. Gamasida (Acari) abundance declined significantly with increasing herbicide dose. Soil biological quality, expressed as the multiplied QBS-Ar index, was significantly reduced at the highest application rate. Soil respiration exhibited the strongest response, declining markedly with increasing fluroxypyr dose and reaching the lowest values at 400 g ha⁻¹.

These results demonstrate that fluroxypyr, at commonly applied agricultural doses, can impair both soil microbial functioning and key decomposer groups. The findings highlight the importance of incorporating non-parametric, community-level and functional indicators—particularly total Collembola abundance and respiration—into herbicide risk assessment. They challenge the assumption that auxinic herbicides pose negligible risks to belowground biodiversity and support the need for more inclusive evaluation frameworks for soil organisms.

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4.14.P-Th188 A Microcosm on Tires? – Investigating the Presence of Microorganisms on Tire Wear Particles in Soil under Different Environmental Conditions.

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It is well established, that tire wear particles (TWP) pose a risk to the environment. Not only are they persistent, but they also contain a complex mixture of substances, such as zinc and other heavy metals, as well as polycyclic aromatic hydrocarbons (PAHs), that can be released into the surrounding environment through leaching. Especially terrestrial organisms are at risk to be exposed to TWPs and their leachables, as soil is the major sink for TWPs. Upon this potential risk for the environment emanating from TWPs, the question arises, if soil microorganisms are still able to grow in such a seemingly hostile environment.

To investigate the growth of soil microorganisms on the surface of tire wear particles, different amounts of TWPs in the size range of 0.8 – 1 mm were mixed with soil samples of varying water content and incubated at room temperature in the dark for several weeks. The TWPs were then viewed in the SEM to examine them for microbial presence at different time points. To enable reliable identification of microorganisms in the SEM, several TWPs that were not mixed with soil were viewed in the SEM and compared with TWP samples that were incubated in soil and subsequently freeze-dried to preserve any attached microorganisms. Additionally, a

leachate of soil samples was prepared in a 0.85% saline solution and filtered through a 10 µm filter to retain microorganisms from the soil. The filter was then viewed in the SEM as well. To determine potential changes in soil microbial growth in the presence of TWPs, DNA was extracted from soil samples exposed to TWPs and from control samples without added TWPs, and DNA concentrations were measured.

Preliminary results indicate that microorganisms are able to grow on the surface of tire wear particles, contrary to our initial hypothesis. However, the identification of microorganisms on TWPs is challenging, as the particles exhibit highly irregular surfaces with agglomeration-like structures and short protruding fibers resulting from the milling process. Furthermore, TWPs can only be used once for SEM analysis, limiting the comparability. As a next step, the experiment could be repeated using environmental SEM (eSEM), which would allow the same TWP to be examined at multiple time points and enable direct tracking of microbial growth. The soil microbiome is an important indicator for soil health, making its monitoring essential for soil research looking to preserve one of earth's most valuable resources.

4.14.P-Th189 Urban Unconventional Water Infiltration and Soil Resilience: Hydrodynamic and Bio-Physico-Chemical Impacts

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Freshwater resources are facing increasing scarcity, which could affect one in four people by 2050 (United Nations, 2015). To deal with these issues, in 2023, France adopted the « Water Plan », which promotes the use of unconventional water (treated wastewater and stormwater) for urban cleaning and green space irrigation.

The study investigates the impacts of unconventional water infiltration on urban soil quality. Soils and unconventional water were characterized for physical (grain size, porosity, density), hydrodynamic (permeability, water retention), geochemical (pH, conductivity, ionic interactions) and biological properties. Non-targeted high-resolution mass spectrometry (HRMS) was used to detect heavy metals, PAHs, pesticides, pharmaceuticals residues and microplastics.

An experimental setup based on soil columns evaluated different soil occupation (vegetated and non-vegetated), soil types and water saturation levels simulating piezometric fluctuations. Hydrodynamic, physico-chemical and microbiological parameters were monitored to follow infiltration impacts, pollutant retention and to better understand the soil – subsoil – groundwater continuum.

Preliminary results reveal strong contrasts: the first soil type exhibits an much higher porosity at depth ($\phi = 70.4 \pm 0.6 \%$), which promotes infiltration, whereas the second soil type is denser ($\phi = 49 \pm 1 \%$). HRMS analyses show over 250 potential features in unconventional water, including aromatics, PAHs, PCBs, pesticides and polar pollutants.

These results highlight the complexity of urban soils and unconventional waters. Integrating hydrodynamic, physico-chemical and “-omics” approaches provides a robust framework to assess soil health and micropollutant retention. This work supports sustainable urban water management and enhances urban soil resilience under increasing environmental pressures.

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4.14.P-Th190 Climate-Induced Remobilization of Nonextractable Organohalogens in Permafrost Soils *Xiaojing Zhu, Helmholtz Center for Environmental Research (UFZ), Germany*

Nonextractable residues (NER) represent a major yet under-recognized portion of organic pollutants in soils. This gap is particularly striking in permafrost environments, where halogenated organic compounds were found to be dominated by NER, frequently exceeding extractable fractions (EF) by two to three orders of magnitude. Such distributions suggest that frozen soils act as large, long-term reservoirs of bound pollutants that conventional EF-based monitoring does not capture.

In this study, NER-to-EF ratios were evaluated across permafrost soils spanning a natural temperature gradient, revealing a consistent decrease in NER relative to EF with increasing temperature. To experimentally

investigate the potential for climate-driven remobilization, controlled incubations were performed on three permafrost soils with contrasting organic matter contents, along with a sterilized control, under a warming–cooling cycle. Across all active soils, temperature shifts led to measurable increases in EF up to 50-fold, indicating mobilization of previously bound organohalogenes. Synthetic-like organohalogenes showed faster release than natural ones, suggesting that NER binding history governs remobilization potential. The magnitude of release varied among soils: higher organic matter contents were associated with slower remobilization, while soils with lower organic matter exhibited more pronounced increases. Microbial activity proved essential, as distinct phyla were associated with enhanced release in each soil, whereas the sterilized control exhibited strongly suppressed remobilization.

Overall, the results indicate that climate warming can promote the remobilization of legacy organohalogen contaminants stored as NER in permafrost. These findings underscore the need to incorporate NER into soil monitoring and resilience frameworks, especially in northern regions undergoing rapid environmental change.

4.15.P Statistics for Setting Endpoints – from Tried and Tested to New and Exciting Methods

4.15.P-Th191 Statistical Analysis of Ecotoxicology Data for Regulatory Purposes: an IEAM Special Series
Raoul Wolf¹, David Fox² and Jannicke Moe³, (1)Norwegian Geotechnical Institute (NGI), Norway, (2)Environmetrics Australia, Australia, (3)Norwegian Institute for Water Research, Norway

Statistical methodologies and tools applicable to ecotoxicology have developed and improved over recent decades. Nevertheless, regulatory risk assessments are still largely based on statistical principles and approaches that are no longer state-of-the-art. SETAC meeting sessions dedicated to statistical methods have witnessed a high level of interest and discussion regarding the progress and applications of statistical methodologies. Many ecotoxicologists have expressed interest in the revision of the 2006 OECD document no. 54 ("Current approaches in the statistical analysis of ecotoxicity data: a guidance to application"). This guidance is no longer considered reflective of contemporary statistical methods or computational platforms used in ecotoxicology, and its revision process is ongoing.

Against this background, a call for papers for a Special Series in Integrated Environmental Assessment and Management (IEAM) was launched in autumn 2024 with manuscript submissions until summer 2025.

The purpose of this Special Series was to provide an overview of the statistical ecotoxicology landscape and reflect on recent developments, processes and opinions. Relevant topics include (but are not limited to): similarities and differences of toxicity metrics (NOEC, BMD, NEC, ECX, NSEC, and more); developments and tools for dose-response modelling; developments and tools in SSD modelling (model averaging, mixture models); Bayesian vs. frequentist approaches; and case studies of novel statistical methodologies highlighting their benefits. We welcomed and encouraged contributions and viewpoints from members of all sectors, including academia, industry and government.

By the time of writing (November 2025), five papers have been accepted for the series, with several more manuscripts under evaluation. In this poster we give an overview of the final series of papers, focusing on the variety of approaches, scientific novelties, and implications for regulatory ecotoxicology and risk assessment.

4.15.P-Th192 The Benefits of Bayesian Statistics: Examples from the Revised OECD Document 54 Annexes

Raoul Wolf¹, Jannicke Moe² and David Fox³, (1)Norwegian Geotechnical Institute (NGI), Norway, (2)Norwegian Institute for Water Research, Norway, (3)Environmetrics Australia, Australia

OECD Document 54 ("Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application"), first released in 2006, is being revised to include Bayesian methods alongside traditional frequentist approaches. The OECD document 54 annexes provide example datasets from OECD test guidelines 201 (algal growth inhibition), 202 (Daphnia acute immobilisation), 211 (Daphnia reproduction), and 215 (fish juvenile growth). Additionally, a new dataset for 236 (fish embryo acute toxicity) was considered.

The revision reflects a broader shift in life sciences toward Bayesian statistics, valued for handling hierarchical, nonlinear, and data-limited scenarios, while offering transparent assumptions, improved uncertainty quantification, and reproducible workflows. Criticisms of Bayesian methods—higher data demand, greater uncertainty, and subjectivity due to priors—will be addressed through intuitive workflows, checklists, and comparisons with frequentist results.

For concentration-response analysis, log-normal, log-logistic, and Weibull type I models were applied, with model choice guided by Bayesian cross-validation. ECX values and credible intervals were derived from posterior distributions. Hypothesis testing used Bayesian hierarchical regression (analogous to ANOVA), with partial pooling to reduce false positives/negatives and significance assessed via posterior draws and the ROPE

principle, yielding NOEC/LOEC values.

Validation included prior-posterior and posterior-predictive checks, plus sensitivity analyses to detect prior-likelihood conflicts. Results showed Bayesian methods robustly produced EC/LCx and NOEC/LOEC values across datasets, with credible intervals automatically capturing uncertainty from data variability, sample size, and priors. While strong priors influenced some parameters, final endpoints remained unaffected.

Overall, Bayesian analysis matched frequentist outcomes in most cases and offered advantages in challenging contexts (e.g., zero-inflation, small samples). The methodology meets regulatory requirements in principle, demonstrating that Bayesian approaches are not more data-demanding, uncertain, or subjective than frequentist ones—and can even reduce uncertainty in some cases. The study encourages scientists and regulators to adopt Bayesian methods for standard ecotoxicity data analysis, highlighting their robustness, transparency, and potential to improve regulatory statistics.

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4.15.P-Th193 FXMATE: Open-Access Statistics for Ecotoxicity Effects MAde Totally Easy

Jochen Zubrod, Eric Bollinger, Dominic Englert and Felix König, Paliner GmbH, Germany

Chemical registration and environmental risk assessment depend on robust statistical evaluation of ecotoxicity test data. Despite the guidance provided, for example by the OECD, inconsistencies in statistical practice persist, often arising from custom code or opaque workflows. These limitations can bias toxicity estimates and complicate the statistical justification of regulatory decisions.

FXMATE is designed to address these issues as an open-access software framework for reproducible, transparent, and standardized ecotoxicological data analysis. The framework implements automated workflows aligned with OECD test guidelines (e.g., 201 and 211), covering data validation, guideline-specific preprocessing, dose-response modelling, and hypothesis testing. While experienced users retain full control over analytical choices, optional constraints and decision guidance are provided to reduce common statistical errors. The framework combines established state-of-the-art modelling approaches, such as bootstrapping and model averaging, with a modular architecture that allows integration of emerging statistical methods as they gain regulatory relevance, including recently proposed hypothesis tests for count data (e.g., CPCAT and Dunnett GLM). For continuous, quantal, and count data, decision trees derived from and extending OECD guidance formalize the selection of appropriate statistical analyses.

By combining guideline-conform statistical methodology with transparent implementations, FXMATE contributes to more consistent and defensible statistical evaluations of ecotoxicity data. This work contributes to ongoing efforts within the ecotoxicological community to modernize statistical practice and ensure that environmental risk assessments remain both reliable and reproducible. As an open-access scientific infrastructure provided entirely free of charge to the scientific community, FXMATE is expected to lower technical barriers, reduce analytical errors, and improve the quality and transparency of ecotoxicological assessments, goals aligning closely with SETAC's mission and good scientific practice.

4.15.P-Th194 Rethinking NOEC Determination for Quantal Data with Cliff Responses: A Critique of the Cochran–Armitage Test and a Proposed Workflow

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For quantal endpoints, the step-down Cochran–Armitage (CA) trend test is a common route to derive NOECs once monotonicity is confirmed. In cliff-edge response patterns, where no or minimal effects at lower doses followed by a sharp increase, the CA test's linear-trend assumption and default asymptotic p-values can yield false-positive LOECs, particularly when replicate structure is mis-specified. Using a *Daphnia magna* acute immobilization dataset (OECD 202) with 0% immobility up to 20 mg/L, a small effect at 40 mg/L (6.7%), and a large effect at 80 mg/L (63.3%), the standard CA procedure selected a NOEC of 20 mg/L (asymptotic $p=0.006$ at 40 mg/L). In contrast, bias-reduced logistic regression, Williams' test, Fisher's exact tests with Holm–Bonferroni adjustment, and an exact CA computed at the replicate level all support a NOEC of 40 mg/L. We investigate two parallel CA adjustments for replicated cliff patterns, trend-consistent overdispersion assessment with Rao–Scott correction and exact CA with replicate-based sample sizes and evaluate their performance alongside logistic regression in an extensive beta-binomial simulation framework using flexible probit-type dose–response curves. Preliminary results generalize the case study, indicating that both CA adjustments

mitigate cliff-driven false positives and align with biologically grounded inference. The proposed workflow improves reliability of regulatory endpoints by reducing artifacts from trend leverage and sample-size misspecification, maintaining power near the smallest effect size considered biologically meaningful (e.g., EC5–EC10), and transparently handling replicated designs.

4.15.P-Th195 Pool or Not to Pool Controls: An Industry Perspective

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Regulatory ecotoxicology needs a consistent answer to a simple question: which control should we compare to treated groups? Right now, the answer varies by guideline, and so do the endpoints.

Alongside negative control, regulatory ecotoxicity tests require a solvent control when a solvent is used to dissolve the test item. OECD guidance, however, is inconsistent on whether to compare test groups to the negative control or the pool of both controls : some test guidelines recommend pooling when no statistical significance between the two is found (e.g., OECD 210, 211, 220, 222, 239), others do not and to use the solvent control for comparison (e.g., OECD 201, 208, 221). Unlike all other guidelines, OECD 231 prescribes negative control when a significant difference is found. OECD TG 54 adds further ambiguity; its flow charts advise pooling when there are no significant differences, while its text warns that small sample sizes (common in ecotoxicology testing (n of 4–8)) may mask true differences and that pooling combines two sources of variability.

We are not arguing with the statistical appropriateness of this procedure but want to highlight a major implication it brings to regulatory science: pooling can increase test power, which may be desirable in some disciplines, but in regulatory science it can cause lower endpoints, which in turn might lack biological relevance. For example, two labs testing the same substance could report different endpoints solely because one used solvent control (doubling control n). This penalizes low solubility substances.

This is especially impactful in aquatic ecotoxicology which still often relies on statistical significance alone, sometimes meaning LOECs are related to effects as small as 1%.

We propose a harmonized approach: use a single control group for comparisons in regulatory studies. This would align statistical practice with regulatory intent, improve cross-study comparability, and avoid penalizing substances based on test design rather than true effect.

4.15.P-Th196 Are lack-of-fit tests fit for purpose?

Pernille Thorbek¹, Lijuan Yan² and Yahya Ghalayini², (1)Apd/EE, BASF, United Kingdom, (2)BASF, Germany

Lack-of-Fit testing is used as a criterion for selecting the best dose-response models in many Test Guidelines and statistical decision schemes. While most people might understand it as a method that determines whether a model adequately describes the relationship between the model and the data, in fact it tests whether the unexplained variation can be explained purely by random variability. Thus, the lack-of-fit test is a rather theoretical approach that may have limited practical value for real-world decision-making. Even if a dose-response model is not perfect (i.e. some discrepancies beyond random stochasticity), it can still be useful for predicting effects reliably. When a lack-of-fit test is used as a cut-off criteria, sometimes it leads to rejection of very well-fitting dose-response models and instead less reliable interpolation methods are used, or it even prevents endpoint derivation altogether. Indeed, other statistical guidelines advocate that lack-of-fit test should not be used in isolation. We recommend that lack-of-fit should not be used as a hard cut-off criterion, but instead be used in conjunction with visual inspection and other assessment criteria such as AIC and significant model parameters.

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All authors work for BASF

4.15.P-Th197 Statistics for visual injury: data types and options for dose-response modelling and species sensitivity distributions

Pernille Thorbek¹, Lijuan Yan², Thomas Sinclair², Christoph Mayer² and Ruth Albuquerque³, (1)Apd/EE, BASF, United Kingdom, (2)BASF, Germany, (3)BASF, United Kingdom

There has recently been an increasing demand for statistics for visual injury generally in the form of EC50 or ER50 and Species Sensitivity Distributions (SSDs). To estimate reliable EC50s and subsequently SSDs, the data have to be suitable for dose response modelling. This would typically mean numerical quantitative data. While visual injury scores may appear quantitative, because they are expressed in percent damage, they are in fact closer to an ordinal scale. In line with this, the OCPPTS 850.4000 series generally considers visual ratings as qualitative data, rather than quantitative. Moreover, a previously published survey shows there are large differences in scoring methods, as well as how these are interpreted both between and within labs. Consequently, while the visual injury data can be ranked, they are not on a continuous scale, e.g. 60 % is not necessarily the same as twice 30%. This represents a violation of the assumptions for most dose-response models typically used in ecotoxicology. Here, we outline different options for dose-response modelling of visual injury data and discuss to what extent each violates the statistical assumptions. Nevertheless, before work is undertaken to change test guidelines and scoring methods for visual injury, it would be prudent to first check whether other endpoints, such as biomass SSDs, are protective of also visual injury.

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4.15.P-Th198 Biological relevance and statistical significance in Collembola soil field studies

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Soil mesofauna field studies conducted for ecological risk assessments of plant protection products (PPPs) often require expert knowledge. In an ongoing debate about alternative statistical methods, the Closure Principle Computational Approach Test (CPCAT; introduced for Poisson-distributed count data) is presented. However, the biological relevance of potential effects in field studies remains undiscussed.

The primary aim was to investigate how to implement biological relevance in data evaluation. Biological Control Ranges (BCR) were established for each taxon and sampling time point within individual studies. Quantified as a percentile of the respective control abundance data to exclude extreme values, the BCR was considered as indicator for biological relevance of potential PPP effects by comparing whether the average abundance of a treatment group is in- or outside of the BCR. High variability in control abundance of Collembola resulted in largely varying BCR values between studies.

The secondary aim was to investigate performance of different statistical methods and their impact on the biological relevance conclusion. Abundance data exhibited over-dispersion in the majority of cases, violating the Poisson-distribution assumption. Consequently, CPCAT showed significant differences in more than 50% of the comparisons between control and treatment plots even before test item application happened in the field (at pre-treatment sampling). Post-application, most statistical significances determined by CPCAT occurred despite the absence of a dose-response relationship and the arithmetic mean abundance values in the assigned treatment plots being within the BCR; thus, they should be considered as false positive results. In contrast, the Dunnett's test and Ranked Dunnett's test which are either considering normally distributed data or independent from the data distribution barely detected a significant difference at pre-application when the abundance average values of assigned treatment plots were within the BCR. Similar treatment-independent observations can be made at post-application samplings. Our investigation shows that the pre-requisites for CPCAT (Poisson distribution, no over-, or under-dispersion of data) were not met in the majority of the field data cases examined.

This poster highlights the importance of a holistic interpretation combining ecological context via BCR with fit-for-data/appropriate statisticals to ensure meaningful conclusions.

4.15.P-Th199 Methods for Assessing Dispersion to Guide CPCAT Use for NOEC Determination

*Lijuan Yan¹, Frank Staab¹ and **Pernille Thorbek²**, (1)BASF, Germany, (2)Apd/EE, BASF, United Kingdom*

CPCAT has been proposed for analyzing field data or soil and other organisms. However, it has also been shown that when data does not follow a Poisson distribution, the results are less reliable as overdispersed data leads to increased rates of false positives and underdispersed leads to increased rates of false negatives.

Therefore, it is important to reliably assess the underlying distribution of the data to be analysed before using CPCAT. The Hampel identifier recommended by Lehmann et al. (2018) does not always give reliable results.

Moreover, a practical challenge to assessing the underlying distribution is the typical limited replication in field studies, which reduces the power of single study dispersion tests. In order to achieve more reliable determination of the underlying distribution, we propose aggregating control group observations across multiple soil mesofauna field studies and assessing dispersion at different taxonomic resolutions. An added advantage of such an approach is that ecological information about how these organisms are distributed in space (and time) can help inform appropriate statistical methods. This poster will (1) summarize limitations of existing dispersion diagnostics for small sample ecotoxicological studies, (2) report empirical dispersion patterns by taxon using pooled control data, and (3) give practical pre screening guidance for CPCAT application. We welcome collaboration and funding to extend this work; preliminary results and a proposed study protocol will be presented.

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All authors work for BASF

4.15.P-Th200 When Counts Deviate: Robust Overdispersion Diagnostics for Ecotoxicity Data

Benjamin Daniels, Pia Kotschik, Susanne Walter-Rohde and Thomas Gräff, German Environment Agency (UBA), Germany

Count-based endpoints such as reproduction or abundance measurements are central to many laboratory and field ecotoxicity studies. Although several statistical procedures for analyzing such data have been proposed, their regulatory use is only sparsely standardized in key guidance documents such as ISO 20281 (2006) or OECD 54 (2006). Robust tools exist, most notably GLM-based Dunnett tests using Poisson, quasi-Poisson or negative-binomial error structures. These approaches combine generalized linear models (GLM) with Dunnett-type multiple comparisons to detect treatment-related decreases or increases relative to a control. An additional Poisson-based alternative is the CPCAT, which similarly depends on the Poisson distribution but incorporates the closure principle as a rigorous framework to control α -inflation.

Yet a key unresolved question is how to evaluate whether Poisson-based assumptions are appropriate for a given dataset. While Poisson models imply equality of mean and variance, real ecotoxicological datasets often deviate from this, especially field reproduction data, which frequently show overdispersion driven by environmental and biological variability.

This raises a practical issue: at what point does deviation from mean-variance equality invalidate Poisson-based test methods for deriving NOECs? Mild departures are expected due to sampling variability and are typically tolerated by established procedures, yet clear guidance on acceptable deviations is lacking. Several diagnostics exist to quantify such departures. The regression-based overdispersion test of Cameron & Trivedi (1990) and the Pearson dispersion statistic are widely used but require fitting a Poisson GLM in advance, thereby inheriting potential model-specification issues and limiting their suitability for early exploratory checks.

In this contribution, we compare the strengths and limitations of these model-based diagnostics using a meta-analysis of existing and simulated data. We further introduce a distribution-agnostic alternative that assesses mean-variance differences directly within treatment groups, allowing both over- and underdispersion to be detected. This method provides an accessible and interpretable tool for standardized preprocessing of count data and supports transparent decision-making when selecting appropriate statistical procedures for NOEC derivation. Our aim is to contribute to a more harmonized framework for analyzing count data in ecotoxicology.

4.15.P-Th201 Old-fashioned data for a contemporary approach: Optimising toxicological studies design for Benchmark Dose modelling.

Michail Gioutlakis¹, Jens Schabacker², **Martin Vallon**¹ and Torben Wittwer¹, (1)RIFCON GmbH, Germany, (2)Rifcon GmbH, Germany

Benchmark Dose (BMD) modelling is an approach for deriving an appropriate endpoint from dose-response data that can be used in environmental wildlife risk assessment. The use of BMD was introduced into the EU regulatory framework in 2005 and since then one scientific opinion and two guidance documents specifically on BMD have been published by the European Food Safety Authority (EFSA). The Guidance document on risk assessment for birds and mammals (EFSA 2023) requires the use of BMD modelling to determine endpoints from toxicity studies data instead of using the No Observed Adverse Effect Level (NOAEL).

The currently used guidelines for toxicological studies have been developed to generate data suitable for NOAEL determination but not for BMD modelling. Although BMD modelling makes a better use of dose-response data, data generated by the current study designs might be unsuitable for BMD modelling or they

might lead to unreliable results that cannot be used in wildlife risk assessments. Even if BMD modelling is required by EFSA 2023, new toxicological studies are avoided for animal welfare reasons.

Optimising number of tested doses, spacing between doses and the number of animals per dose within the framework of current OECD and EPA guidelines (OECD 206, 416, 443, 414 and USEPA 850.2300) could improve data suitability for BMD modelling and consequently the reliability of the results in cases where testing is permitted.

A review of the available guidance documents, scientific opinion and the relevant scientific literature was performed, and specific recommendations can be given for optimising the study design of birds and mammals toxicological studies in favor of BMD modelling. Furthermore, characteristics of a design that can lead to unsuitable data will be presented.

The resulting recommendations can be considered for planning toxicological studies, that are intended to be used for BMD modelling. With our study, we contribute to the discussion about revision of study designs within guidance documents to generate data suitable for BMD modelling.

4.15.P-Th202 Challenges of Scientific Rigor and Practical Implementation of EFSA Scientific Opinion on The Use and Reporting of Historical Control Data

Zhenglei Gao¹, Stefan Kimmel², Felix Kluxen³ and Emily Scorgie⁴, (1)Bayer AG, Germany, (2)Corteva, Germany, (3)BASF, Germany, (4)Syngenta, Netherlands

Historical control data (HCD) are an important element in toxicological evaluations with emerging importance in ecotoxicology. Currently, researchers typically consider HCD only after conducting the study analysis, using them to contextualise results via expert judgement. A recent EFSA scientific opinion introduces a novel approach of incorporating HCD into the statistical analysis itself. Put simply, the idea is to combine concurrent control (CC) data with a curated subset of HCD to create a combined control group for use in the (likely more complex) statistical analysis. This approach promises a less subjective use of HCD, but raises methodological and operational challenges. The key issues include defining exchangeability between CC and HCD, pre-specifying HCD inclusion criteria, and choosing modelling frameworks that borrow information from HCD adaptively while controlling error rates. We explore the soundness of the proposed novel approach along with anticipated challenges regarding practical implementation, if the Scientific Opinion were to be developed into guidance for regulatory processes.

4.15.P-Th203 Assessing LD50/10 as a Proxy for Avian Reproductive Risk in Wildlife

Jens Schabacker¹, Martin Vallon² and Michail Gioutlakis², (1)Rifcon GmbH, Germany, (2)RIFCON GmbH, Germany

According to the 2023 EFSA guidance document on risk assessment for birds and mammals, the acute toxicological endpoint tested, divided by ten, should be used as a surrogate toxicological endpoint for reproductive risk assessment when it falls below the reproductive endpoint. This idea originates from a 2008 EFSA publication, specifically Appendix 11 (Callahan & Mineau 2000), in which sublethal clinical signs (such as hypo-reactivity, asthenia, anorexia, ataxia, emaciation and piloerection) were analysed in 166 acute avian pesticide studies. The aim of the study was to identify humane test designs that could predict LD₅₀ values.

The LD50/10 concept involves extrapolating acute lethality data to potential reproductive impairments, such as disrupted pair formation or parental care. However, it should be noted that the assumption that acute toxicity reliably predicts long-term, population-relevant effects on reproduction has not yet been proven.

In the Avian Acute Oral Toxicity Test (OECD TG 223), a single high dose being administered by gavage to fasted birds, producing rapid peak plasma concentrations and immediate toxic effects. Although these tests are useful for identifying hazards, they do not represent realistic environmental exposures, which are typically lower and continuous.

In contrast, the Avian Reproduction Test (OECD TG 206) involves monitoring the entire reproductive cycle to determine endpoints such as fertility, hatchability, and offspring survival following dietary exposure. These endpoints are considered to be more ecologically relevant. According to the 2023 EFSA guidance document, the biologically relevant effects should be chosen for the endpoint setting of wild birds: e.g. direct reproductive and developmental effects; body weight changes of at least 10% (BMDL10); and behavioural or neurological effects

affecting survival or mating. Other effects, such as organ-level, minor, or transient effects, are generally considered to be less relevant.

Although the sublethal signs identified by Callahan and Mineau are useful for their intended purpose, they do not correlate with population-level reproductive effects, which limits their ecological relevance. Consequently, while the use of LD₅₀/10 as a surrogate endpoint in ecological reproductive risk assessments is possible, it is not always appropriate, as it can overestimate, underestimate or misrepresent the true reproductive endpoint. Unfortunately, this is not stated in EFSA (2023).

4.16 Towards a Fit-For-Purpose Risk Assessment of Non-Conventional Plant Protection Products

4.16.T-01 Formulation-Dependent Cellular Bioavailability of dsRNA in Non-Target Organisms: Implications for Environmental Risk Assessment of RNA Interference Based Pesticides

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RNA interference (RNAi)-based pesticides are a novel approach for crop protection, offering a species-specific mode of action distinct from conventional chemical and biological pesticides. These products use double-stranded RNA (dsRNA) molecules designed to match to essential mRNA sequences in target organisms. Upon uptake, the dsRNA activates the organism's RNAi pathway, leading to degradation of the target mRNA, which can result in impaired growth, reproduction, or survival.

Due to their sequence specificity, RNAi-based pesticides are expected to pose minimal risks to non-target organisms (NTOs), which potentially contributes to developing more sustainable plant protection products. Nevertheless, there are still key uncertainties regarding environmental risks, especially in relation to exposure, off-target effects and cellular bioavailability, which is influenced by the formulation of the dsRNA. Many pests still cannot be targeted through RNAi due to biological barriers and the low persistence of naked dsRNA. Consequently, substantial research is focused on formulations that increase the persistence and bioavailability of dsRNA. Although prolonged persistence and cellular bioavailability improves pest control, it also increases environmental risks and may affect the susceptibility of NTOs. Therefore, the changed properties of formulated dsRNA must be urgently addressed in environmental risk assessment.

The aim of the first phase of our research project is to establish a test system to evaluate the cellular bioavailability of formulated dsRNAs in standard NTO models, focusing on the zebrafish embryo model first. To achieve this, zebrafish embryo tests were conducted in accordance to OECD guideline 236 using sublethal concentrations of the formulated dsRNA to enable downstream analysis. To establish a standardised positive control protocol, embryos of the transgenic zebrafish line Tg(fli1:EGFP) were exposed to a nano-lipid formulated dsRNA targeting the enhanced green fluorescence protein (EGFP) messenger RNA. EGFP downregulation was assessed both at the protein level by quantifying the fluorescence signal as well as at the mRNA level using quantitative polymerase chain reaction (qPCR) and transcriptome analysis. Together, these complementary approaches are expected to provide a comprehensive basis for demonstrating the suitability of using EGFP transgenic NTOs to test the cellular bioavailability of RNAi-based pesticide formulations

4.16.T-02 A meta-analysis of peptides used in plant protection: Sources, modes of action, and structure-derived properties of regulatory importance

Nina Cedergreen and Kitzia Molina-Zamudio, No Affiliation, Denmark

Many chemical plant protection products (PPPs) pose risks to human health, non-target organisms, and the environment, prompting a shift toward natural alternatives. Peptides have gained attention as promising candidates due to their low environmental persistence, target specificity, and diverse mechanisms, acting as antimicrobials, insecticides, antivirals, anthelmintics, and herbicides. Despite increasing recognition, peptides are not yet fully integrated into the PPP landscape, with a lack of systematic classification and risk assessment frameworks.

This meta-analysis compiles current knowledge on peptide-based PPPs, including those that are

commercialized, patented, or under development, and proposes a clear categorization based on structural and physicochemical features. We compile our dataset by extracting peptides from references in Web of Science, antimicrobial peptide databases, and superfamily classifications, ultimately yielding 4,492 unique peptides after deduplication and filtering for only plant protection.

Based on our database, the most common use of peptides is antibacterial, followed by antifungal, insecticidal, and antiviral. However, the intended uses commonly overlap, with 61% of peptides claiming broad-spectrum activity, raising concerns about non-target effects. Therefore, instead of classifying them by use, we analysed their structural architecture and physicochemical properties and correlated them with the reported modes of action. Generating peptide clusters with specific structural and physicochemical properties facilitates the formulation of hypotheses on environmental effects and their impacts on beneficial organisms.

This peptide database will form the foundation for our work in ENSAFE (ENSAFE.dk), where our aim is to provide data and models to predict the environmental fate and effects on non-target organisms of exemplary peptides.

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4.16.T-03 A Fit-for-purpose Approach for the Assessment of Active Substance of Low-Concern

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The EU regulatory framework for pesticide risk assessment was primarily conceived for conventional synthetic pesticides and does not consider the specificities of Low-Concern Active Substances (LCASs), such as botanicals, semiochemicals, microbial metabolites, inorganics, peptides, and dsRNA. In addition, some of the current data requirements may not be needed for these types of substances, if adequately justified. However, without agreed guidance on how to identify the relevant data to be submitted and how to perform the environmental risk assessment (ERA) for these types of substances, dossiers for LCASs end up with many data gaps. Furthermore, concerns have been raised as to whether alternative modes of action are appropriately addressed with the standard data requirements and ERA.

To address these challenges, a more flexible and fit for purpose ERA approach was needed, that would allow both a better justification of data waiving and to take into account the particular properties of the active substance.

This approach was developed by a consortium in response to a call launched by EFSA in 2024. Based on problem formulation, the proposed approach makes use of pathways to harm (PtH), where harm is defined as not meeting the protection goal. A PtH is defined as a sequence of events through which a hazard can lead to adverse effects. In this context, the aim of the PtH is to assess the likelihood of breaching the protection goal, based on the likelihood of each individual event to take place. The PtH are in this way used as a method to combine all lines of evidence given by the data available for an overall weight-of-evidence assessment. Detailed, generic PtH for each relevant hazard (toxicity, suffocation, desiccation, hypoxia, RNA interference, mating disruption, leaching potential) and non-target organisms' (birds and mammals, aquatic organisms, bees, non-target arthropods, soil macro-organisms, soil microorganisms, non-target terrestrial plants) combination have been developed. In addition, analysis plans providing information on which analyses may be used to assess the likelihood of the individual events were built. Further, guidance on the likelihood assessment, including the uncertainty analysis related to this assessment, is given.

This presentation will present the approach developed, its added value and how we believe it will allow to address (part of) the challenges encountered when assessing such actives substances.

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4.16.T-04 Advancing Ecotoxicological Risk Assessment for Microbial Plant Protection Products: Insights from RATION WP2.2

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The increasing use of microbial plant protection products requires robust ecotoxicological risk assessment frameworks tailored to their unique biological characteristics. EU regulations mandate comprehensive environmental safety evaluations, yet standard testing protocols designed for chemical pesticides may not adequately address microbial-specific traits such as viability, colonization potential, and biological mode of action. RATION WP2, Task 2.2 developed a tiered ecotoxicity testing strategy and problem formulation (PF) tailored for microbial pesticides.

A systematic 4-step approach was implemented using 3 model microbial active substances (SeMNPV BV-0004, *B. velezensis* FZB42, *P. lilacinum* PL11): PF through literature and regulatory assessment; adaptation of OECD guidelines; proof-of-concept testing; and integration into risk assessment development. Standard protocols were modified to address microbial characteristics, including natural soil with native communities, repeated applications mimicking GAP practices, extended pollinator exposure periods, and sensitive life stage assessment. A harmonized analytical strategy combining DNA detection via qPCR with viability assessment was applied across environmental compartments.

Problem formulation identified specific hazard and exposure considerations including viability under field conditions, colonization potential, sensitive life stages, and realistic exposure routes. Adapted protocols enabled microbial-appropriate risk characterization. Experimental results provided empirical data across terrestrial, pollinator, and aquatic compartments. In soil, PL11 and FZB42 showed no significant effects on earthworms, Collembola, or nematodes. For pollinators, acute and chronic tests showed no adverse effects at field rates, though PL11 caused larval mortality at 10× maximum field rates, identifying honey bee larvae as a sensitive life stage. Aquatic microcosm studies with FZB42 showed no significant effects on invertebrates or algae. The qPCR-viability strategy is proving robust for differentiating living from non-living microbes.

Integration into RATION WP2, Task 2.4 framework is creating a feedback loop where experimental data update problem formulation and inform scenario selection and waiver criteria. This work is providing methodological tools and scientific evidence to inform EU regulatory frameworks, ensuring microbial PPPs are assessed with ecological relevance while minimizing unnecessary testing.

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4.16.P Towards a Fit-For-Purpose Risk Assessment of Non-Conventional Plant Protection Products

4.16.P-Mo320 Bio-pesticides: A Risk Assessor's Nightmare or an Opportunity to Re-Wire the Risk Assessment?

John Kandris, **Sofia Mirmigkou** and Katerina Kyriakopoulou, Benaki Phytopathological Institute (BPI), Greece

It is apparent in recent years that non-conventional active substances have become popular in plant protection. Substances such as plant extracts enter the market with the hope of taking their share in the market against "traditional" active substances. However, these non-conventional substances (e.g. of botanical origin, substances of Unknown or Variable Composition, Complex Reaction Products, or Biological Materials; UVCBs) fall under the same data requirements as the conventional chemicals used in plant protection (Reg.1107/2009) which

implicates the environmental risk assessment.

Issues challenged with their identity, difficulties with deciding the appropriate substance to be considered relevant for the environmental risk assessment, are only some faced by risk assessors and can potentially impact the exposure assessment when dealing with such dossiers. In addition, issues concerning the fulfillment of the data requirements according to Reg. 1107/2009, 283/2013 and 284/2013 are also apparent as they picture a conventional and strict assessment with the provisions of specific tests concerning a synthetic active substance compound.

Examples of such substances are capric, caprylic and pelargonic acid, paraffin oils, fatty acids from hydrolyzed vegetable oils. In these cases, alternative approaches were followed for the exposure assessment and testing, with the most apparent being the read-across between dossiers based on chemical similarity, the work around which needed to be decided as the characterization of their composition is not possible, and, the use of QSAR estimates for environmental properties, although they are not recommended for risk assessment, since are not reported in any EU guidance document or communication list. In addition, data requirements have been waived based on lead components' natural occurrence in the environmental matrices, indicating the non-feasibility of testing.

These different approaches might be a potential and effective way forward to the environmental risk assessment under Plant Protection Product regulatory framework. Using available information from open literature, such as natural occurrence in the environment, DT50 values for PEC calculations a more pragmatic approach is introduced. In combination with a read across approach through similar dossiers and a well-studied ecotoxicological profile, safe use for specific bio-pesticides might be demonstrated even without a quantitative risk assessment.

4.16.P-Mo321 Science-based data waivers for low-concern pesticides: A Pathways-to-harm Problem-formulation Toolbox

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Low-concern pesticides such as microorganisms, botanicals, semiochemicals, inorganic substances and biomolecules are increasingly promoted to reduce dependence on synthetic pesticides. Their diverse physicochemical properties, modes of action (e.g., suffocation, mating disruption, RNA interference) and application patterns challenge current EU risk-assessment schemes, which were mainly designed for conventional toxicants applied by foliar spraying. To address this gap, we developed a fit-for-purpose problem-formulation (PF) approach using generic Pathways to Harm (PH) and linked analysis plans, with a specific focus on science-based data waiver criteria. Waiver criteria were systematically collected from EFSA peer-review conclusions and Rapporteur Member State reports, relevant European Commission and OECD guidance, read-across from non-EU frameworks (EPA, Canada, Australia), and targeted case studies for botanicals, straight-chain lepidopteran pheromones, dsRNA, peptides, microbial metabolites and inorganics.

The criteria were grouped by LCAS type. Typical justifications include exposure comparable to natural background levels, rapid breakdown to non-toxic products, high volatility and low persistence, food-grade or naturally ubiquitous substances with a history of safe use, and inert, insoluble inorganic minerals.

These grouped criteria were then integrated into a spreadsheet-based Analysis Plan tool consisting of seven hazard-specific sheets (toxicity, hypoxia, suffocation, desiccation, mating disruption, RNA interference and leaching), each aligned with generic PH events and providing structured options for evidence generation, including NAMs.

The resulting toolbox supports transparent, harmonised and efficient regulatory assessments for existing and emerging low-concern pesticides while maintaining EU environmental protection goals.

Disclaimer/Disclosure:

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4.16.P-Mo322 Streamlining risk assessments using Decision Trees for biocontrol products

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The growing global demand for sustainable agricultural practices has led to a growing interest in biocontrol products as viable alternatives to conventional chemical pesticides. To effectively regulate these biological products, experts from member companies of the International Biocontrol Manufacturers Association (IBMA) have developed decisions trees which support identification of potential risk areas for natural substances and microorganisms when used as plant protection products.

The first data decision tree categorizes natural substances into three groups based on their identification and characterization and their known or suspected risk profiles. The data decision tree on natural substances, introduces a tiered approach to identify and assess potential risks in four key areas: effects on human health, residues, environmental fate and behaviour, and effects on non-target organisms. For each group, the use of the decision tree for natural substances allows to reflect on the needed data to assess human and environmental safety.

The second decision tree addresses the challenges associated with the evaluation of microorganisms as biocontrol agents. Starting with the identity of the microorganism, its biology and ecology can be described and used to assign it to one of six ecological groups. For these groups, proposals are made which information is needed to determine the risk to humans through direct exposure or residues, to non-target organisms or the environment. The potential production of microbial compounds is included in the decision tree, and a proposal is made which compounds need special attention.

A third decision tree about semiochemicals has been submitted for publication in a peer-review journal.

All decision trees are designed to support a more efficient and science-based regulatory process, facilitating the market access of biocontrol products while ensuring human and environmental safety. They align with current ongoing EU initiatives such as “pathway to harm” and the “problem formulation approach” endorsed last year. These tools are intended for use by regulatory authorities, researchers, and industry stakeholders to accelerate the adoption of sustainable pest management solutions.

This presentation will provide an overview of the decision trees, highlighting their structure, key considerations, practical applications in regulatory submissions and their role in advancing sustainable agriculture globally.

Disclaimer/Disclosure:

Related publications (disclaimer mentionned in each of the publications):

Data decision tree for identifying potential risks for natural substances when used in plant protection:
<https://www.tandfonline.com/doi/full/10.1080/09583157.2023.2210268#abstract>

Data Decision Tree for identifying potential risks for microorganisms when used in plant protection:
<https://www.tandfonline.com/doi/full/10.1080/09583157.2025.2450414?src=>

4.16.P-Mo323 Towards a Fit-For-Purpose Risk Assessment of Non-Conventional Plant Protection Products in the TRIBIOME Project

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Non-conventional plant protection products (NCPPs), such as microbial formulations and plant extracts, are promising sustainable alternatives to synthetic pesticides. However, regulatory frameworks and risk assessment methods, designed for conventional chemicals, are often ill-suited for these novel substances. The TRIBIOME

project addresses this gap by developing fit-for-purpose risk assessment strategies for NCPPs, particularly those designed to modulate the plant-soil microbiome.

The project's methodology involved a comprehensive safety evaluation of specific microbial strains and plant-derived extracts (cane and onion). This included assessing their efficacy in enhancing plant resilience and nutrient uptake. A core component was an extensive toxicological testing regime using in vitro cell viability assays on human lung (A549), skin (HaCaT), and intestinal (CaCo-2) cell lines, and ecotoxicity evaluations on freshwater biota (algae and *Daphnia magna*). The project also advocates for an adapted risk assessment framework that incorporates New Approach Methods (NAMs) and predictive modeling to reflect the unique, often lower-risk properties of NCPPs.

The results demonstrated a strong safety profile. Neither the plant extracts (0–100 ppm) nor the microbial strains (10^3 – 10^7 CFU/mL) induced cytotoxicity in any human cell models. In aquatic tests, all microbial agents and both plant extracts showed no adverse effects on algal growth. However, *Daphnia magna* exhibited differential sensitivity, with cane extract showing higher toxicity (LC50 of 1.24 ppm) than onion extract (LC50 of 24.6 ppm), indicating that certain plant extracts require concentration-dependent risk management for aquatic invertebrates.

In conclusion, the TRIBIOME project confirms that the assessed NCPPs, especially microbial strains, present minimal risks to human health and primary producers. While some plant extracts require careful consideration for aquatic invertebrates, the overall findings underscore the necessity of developing tailored regulatory frameworks that facilitate the safe integration of these sustainable products into agriculture, moving beyond one-size-fits-all chemical pesticide models.

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4.16.P-Mo324 Regulatory Strategies for E-Fate and Ecotox Assessment of Natural Substances in Plant Protection

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Natural substances and semiochemicals are increasingly used in plant protection and integrated pest management due to their favorable environmental profiles. This trend reflects a reduction in chemical pest management and a growing emphasis on alternative, innovative pest control solutions. In the EU, a key challenge is meeting the requirement for standardized testing for environmental fate (e-fate) and ecotoxicology (ecotox) for these substances.

For naturally occurring compounds that are readily biodegradable, e-fate and chronic ecotox studies are unnecessary. When biodegradability is uncertain or limited, testing may be required. This poses challenges during study design due to characteristics including mixture complexity, variable components, inorganic nature, insufficient radiolabelling possibilities, high volatility, low solubility, or hydrophobic properties. These features often cause technical difficulties in dosing, measurement, and create significant obstacles to interpretation.

A tiered approach is proposed to determine testing needs:

First tier: A weight-of-evidence strategy, as for microorganisms, integrating existing data from EU frameworks (e.g., PPP Regulation, REACH), biodegradation testing, QSAR, and read-across from similar substances.

Expanded guidance on read-across for environmental risk assessment would enable harmonized workflows based on structural similarity and toxicity profile alignment, consistent with OECD and EFSA principles.

Second tier: Focus on identifying compartments of concern and relevant non-target organisms. For substances with limited chronic bioavailability, acute exposure should be prioritized. Pragmatic PEC modelling can be used to identify compartments that require further assessment.

Third tier: Fit-for-purpose testing involves adapted designs, such as closed systems for volatile compounds, passive dosing for low-solubility substances, or tailored analytical methods. The focus should be on compartment-specific testing and assessment of relevant non-target organisms.

Aligned regulatory flexibility to accept robust waivers or non-standard methods is essential for registering substances with unique properties. The tiered approach ensures environmental safety with strong protection goals, supports innovation and EU policy, while avoiding unjustified testing burdens which may restrict market access for innovative compounds.

4.16.P-Mo325 Using problem formulation for an efficient, fit-for-purpose risk assessment of microbial plant protection products

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Biological plant protection products (PPP) are important building blocks for integrated pest management and can contribute to a more sustainable agriculture. In Commission Regulation (EU) No 283/2013 microbial PPP are referred to as PPP containing an active substance that is a microorganism. Before these products can be placed on the market, the environmental and public health safety of their use need to be assessed. In other regulatory frameworks, such as for GMOs, also the release of microorganisms is assessed. Similarly to this regulatory framework we propose to use the problem formulation approach based on pathways to harm to arrive at a fit-for-purpose environmental and public health risk assessment of microbial PPP (i.e., both the active substance and the product in the context of the European PPP regulation), by tailoring the risk assessment to the individual microbial PPP and harmonizing this approach when possible. Pathways to harm are being developed for different hazards, among others antimicrobial resistance, invasiveness, toxicity, pathogenicity and infectivity. Each pathway to harm consists of several events, starting with the use of the PPP and ending with the protection goal. Testable hypotheses are formulated for each of the events in the causal pathway to harm. For each testable hypothesis, the information is identified which is needed to test it. Each of the events in the pathway has to take place for harm to occur, therefore it is efficient to start with the event in the pathway which is easiest to assess. This approach is flexible, efficient and can be adapted to the microorganism in question or the use which is being assessed. In case none of the events can be excluded to occur, the pathway to harm approach helps to combine all available information in a weight-of-evidence approach to assess the likelihood of not meeting the protection goal. By providing generic pathways to harm, the approach also helps to harmonize assessments while allowing for flexibility. Problem formulation also leads to a more transparent assessment and helps to communicate about risks. Hence, risk managers are better informed and the resulting risk management decision can be better substantiated.

4.16.P-Mo326 Ecotoxicological Data Gaps in EFSA Reviews of Microbial Plant Protection Products: A Focus on Secondary Metabolites and Infectivity/Pathogenicity

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Microbial active substances provide environmentally friendly alternatives or complements to chemical pesticides for sustainable agriculture. However, their regulatory assessment presents distinct challenges: once released, microbial agents may produce secondary metabolites, multiply, spread, and potentially transfer antimicrobial resistance genes, necessitating tailored risk assessment approaches fundamentally different from those for chemical pesticides. Regulatory approval faces recurring ecotoxicological data gaps that prolong evaluation periods. Understanding predominant concerns and common deficiencies is essential for streamlining dossier preparation and ensuring robust risk assessments.

We systematically analysed publicly available European Food Safety Authority (EFSA) peer review documents for microbial active substances in the EU Pesticides Database, focusing exclusively on ecotoxicological issues. The dataset captured 126 microbial active substances (fungi, bacteria, viruses), yielding 1,078 observations categorized by EFSA classification (data gap, not finalized, critical concern, no issues), area of concern (pollinators, soil organisms, aquatic organisms, birds and mammals, non-target arthropods), and keywords (secondary metabolites/toxins, infectivity/pathogenicity, data missing). We quantified frequencies and identified patterns by microbial type and environmental compartment.

The majority of issues were data gaps (52%) or not finalized risk assessments (48%). Pollinators represented 24% of concerns, followed by soil organisms (21%), aquatic organisms (19%), non-target arthropods (17%), and birds and mammals (15%). Two dominant themes emerged: secondary metabolites/toxins (45% of issues) – including uncertainty regarding production levels, environmental fate, and toxicity to non-target organisms; and infectivity/pathogenicity (24%) – requiring data on potential infection across life stages and clearance assessments. Notably, 55 of 86 substances were classified as not low risk.

Recurring ecotoxicological data gaps, particularly concerning secondary metabolites and

infectivity/pathogenicity, significantly challenge regulatory approval across all non-target organism groups. These findings provide actionable insights for industry stakeholders to anticipate regulatory requirements, prioritize data generation, and accelerate market authorization while ensuring environmental safety.

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4.16.P-Mo327 Improving Aquatic Toxicity Tests with MPCA's – How, When, and at What Levels to Test **Henry O Krueger¹ and Sarah McCoy², (1)Senior Advisor Eurofins Agrosciences Services (US), United States,** **(2)Eurofins Agroscience Services US, United States**

An environmental risk assessment is a function of hazard and exposure. There is currently a lack of modeling and studies to characterize an aquatic exposure via MPCAs, which are vastly different than conventional chemicals. Preliminary work for exposure assessments would be useful and informative when setting test levels. Current guidelines set upper limits of test concentrations in water at unrealistically high levels. These concentrations may be relevant for products that are directly sprayed to water for mosquito control or invasive aquatic plants, but are not realistic for products directly sprayed to fields, soil incorporated, in-furrow applications, or seed treatments. Testing such high concentrations in water can result in opaque test solutions and excessive particles suspended in water that can interfere in the feeding of invertebrates and clog gills in fish. Aquatic testing of conventional chemicals has well defined upper limits for testing; 100ppm or the limit of water solubility. Alternatively, MPCA's are often particles suspended in water and solubility limits are not applicable. Assessing particle distribution in water is more relevant; are they floating, sinking, or in suspension in the water column. Consideration of cell viability, dormancy and metabolite expression in water is also important. We suggest several shorter and less expensive trials to address some of these questions. Preliminary testing more appropriate for MPCA's would be; distribution of particles in the water column, cell viability and expression, as well as effects of light, temperature, pH and salinity. These types of preliminary trials can be useful tools for better understanding exposure that then can be used to design better aquatic tests. The information is also useful in providing data that can be used to support waiver requests.

4.16.P-Mo328 The Effects of a Microorganism-based Plant Protection Product on Zooplankton Community Composition

Judith Epping¹ and Paul van den Brink², (1)Wageningen University and Research (WUR), Wageningen, Netherlands, (2)Wageningen University and Research (WUR), Netherlands

Microorganism-based plant protection products are a promising alternative to synthetic pesticides. Because of the natural occurrence of many microbial strains in the environment and their attributed species specificity, their direct toxicity to aquatic non-target organisms is expected to be low. Yet, information on their fate in water and sediment, including viability, as well as indirect effects on aquatic environments, including zooplankton community composition, is still very limited. For example, it was reported that microbial pesticides may increase turbidity, which could affect primary production or filter feeding, both of which influencing zooplankton fitness. We conducted an indoor microcosm experiment over 28 days with three different treatment concentrations of the microbial pesticide RhizoVital42 (*Bacillus velezensis* FZB 42). Treatment concentrations were 1x application of the max. recommended dose (Day 0), 4x application of the max. recommended dose (Day 0, 7, 14, 21) and 1x application of 10x of the max. recommended dose (Day 0). The set-up consisted of 4 replicates per treatment and 6 control replicates. Microcosm systems included 12L unfiltered pondwater, macrophytes, freshwater shrimp and snails, natural sediment and a natural zooplankton community (collected on Wageningen University campus). Zooplankton samples were collected weekly and identified to lowest taxonomic level possible. Preliminary results indicate that zooplankton community composition in the treatments differed significantly from the controls on day 14, 21 and 28. Further analysis will focus on treatment and species differences and relate the outcomes to results of the currently ongoing quantification of *B. velezensis* in water and sediment over the experiment duration. The results of this microcosm experiment will provide new insights into the effects of microorganism-based plant protection products on aquatic ecosystems and zooplankton communities in particular.

4.16.P-Mo329 Identification of Common Pain Points in the EU Botanical Biopesticide Approval Process: Causes, Consequences and Potential Solutions

Judith Epping¹ and **Tobias Pamminer²**, (1)Wageningen University and Research (WUR), Wageningen, Netherlands, (2)Early Environmental Safety, Bayer AG, Monheim am Rhein, Germany

Biopesticides are considered a promising tool in the ongoing search for sustainable plant protection alternatives. Compared to other industrial countries, the number of approved biopesticides in the EU is relatively low, especially for botanical (plant-based) biopesticides. Botanical biopesticides currently fall into the same regulatory category as well characterized, pure synthetic pesticides, while frequently containing multiple natural compounds of varying concentrations. This poses a challenge to risk assessors and leads to a lengthy and costly registration process, hindering market access of these more environmentally friendly solutions. To address this challenge and improve the requirements for botanical biopesticide registration, it is crucial to know where mismatches with the current scheme occur. We have conducted a structured, semi-quantitative analysis of EFSA peer-reviewed reports on botanical biopesticides to identify frequently flagged issues. Using network-analysis, we determined common connections between unresolved issues and sections in the evaluation dossier with the goal of formulating concrete interventions that could advance the regulatory process for these substances without diminishing safety assessment. We collected information from data gaps that were flagged by EFSA in the review process for 14 botanical biopesticides and assigned keywords to each flagged issue to characterize reoccurring pain points. We also paired keywords to help uncover patterns where issues may propagate throughout the review process. Finally, we ran a co-occurrence network analysis on the top 15% most frequently occurring terms and determined how frequently each keyword occurs as well as which pairs of keywords commonly occur together within each evaluation. Data gaps categorized under the keywords “analytical method” and “technical properties” were most frequently (co-)occurring and are central to the network, meaning they are likely to trigger an issue in a following dossier section. Most flagged data gaps stem from the identity section of the dossier, highlighting the mismatch between the risk assessment requirements and the inherent characteristics of botanical biopesticides. Based on our findings we will provide practical recommendations to start addressing identified issues. We hope this semi-quantitative approach can serve as a blueprint to improve data driven policy analysis in a wide range of highly regulated products beyond plant protection.

4.16.P-Mo330 Study for the Control of Leaf-Cutter Ants, Aiming to Improve Agriculture, Aid Human Health, and Contribute to Ecotoxicology.

João Batista Fernandes¹, **Lulliana Franco¹**, **Eduarda Oliveira dos Santos¹**, **Artur da Silva Cruz¹**, **Anderson do Rego Pires¹**, **Sean dos Santos Araujo¹**, **Moacir Rossi Forim¹**, **Maria Fátima das Graças Fernandes da Silva¹**, **Marisa Narciso Fernandes¹** and **Odair Correa Bueno²**, (1)Federal University of São Carlos (UFSCar), Brazil, (2)Center for the Study of Social Insects, UNESP Rio Claro, Brazil

Omics data, TK-TD (TKTD) models, GUTS, PBK, NAMs, and ADME, studies involving natural products, ants, symbiotic fungi, acetylcholinesterase, environment, toxicology have received great attention contributing to ecotoxicology and to improving the health of the population.

The aim of this study was controlling the leaf-cutter ants (*Atta sexdens*) and their symbiotic fungus, *Leucoagaricus gongylophorus*, to improve agricultural production by evaluating the interaction among natural products from Brazilian plants.

The plants studied were collected in various locations in Brazil, their extracts were obtained using organic solvents and fractionated using different types of chromatography. The identification of the compounds was carried out using modern spectrometry methods, mainly UHPLC-QTOF/MS-MS and NMR. The extracts and isolated compounds were tested as insecticides against ants, for inhibition of fungus growth and the acetylcholinesterase enzyme of *Atta sexdens*.

In this study, the plants *Himatanthus articulatus* (Apocynaceae), *Parahancornia amapa* (Apocynaceae), *Vismia guianensis* (Hypericaceae), *Nectandra rigida* (Lauraceae), *Pouteria torta* (Sapotaceae), and *Clibadium surinamense* (Asteraceae) were used. The prepared extracts of these plants and several isolated compounds showed high insecticidal activity and inhibition of the fungus and in microelectrode of acetylcholinesterase enzyme from *Atta sexdens*.

The identified (28) or noted (36) compounds belong to the following classes: flavonoids, coumarins, chromones, terpenes, pterocarpanes, aryl-naphthalene lactones, iridoid lactones, triterpenes, benzophenones, anthranols, and anthraquinones, such as justicidin B, catechin, pumieride, isoplumieride, plumericin, beta-sitosterol, stigmaterol, citrate of lupeol, beta-amyrin cinnamate, ursolic acid and several inedited compounds. The panel to be presented during the meeting, SETAC EUROPE 36th ANNUAL MEETING, will include the identified compounds, their spectroscopic identification, and the results of the bioassays.

In conclusion, the results obtained contributed to the control of leaf-cutter ants and, consequently, to the improvement of agriculture, assisting in human health and ecotoxicology.

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Keywords:

Natural Products, Ants, Symbiotic Fungus, Acetylcholinesterase, Environment, Toxicology.

4.16.P-Mo331 RNAi-based pesticides - A novel class of pesticides with a huge potential for sustainability requires a novel approach for environmental risk assessment

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RNAi-based pesticides could provide a far more sustainable solution compared to classical chemical pesticides. They are very specific, rapidly degraded in the environment and their active ingredients are naturally occurring substances.

As their mode of action differs significantly from those of chemical synthetic pesticides, they require a different kind of risk assessment.

Current guidelines for risk assessment are designed for the assessment of chemical pesticides. As two RNAi-based plant protection products and one biocide already have entered the EU review procedure for authorization, we are developing approaches for an appropriate risk assessment guideline parallel to this procedure, comprising a tiered alignment analysis using all possible combinations of in-silico-cut siRNAs from the precursor dsRNA against genomes or transcriptomes of non-target species of increasing evolutionary distance from the target organism, and a bioavailability assay. With these approaches the aim is to adapt risk assessment to and possibly reduce data requirements for RNAi-based pesticides in the future, as specific ecotoxicology tests may be waived.

This talk shows why RNAi-based pesticides can be an excellent sustainable alternative compared to chemical pesticides, why they require a different kind of risk assessment, and presents approaches how this assessment can be implemented.

Disclaimer/Disclosure:

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4.16.P-Mo332 Considerations for nucleic acid measurements in soil and sediment systems to meet environmental fate testing guidelines

Hannah Stewart¹, Sarah McCoy¹ and Joshua R. Arnie², (1)Eurofins Agrosience Services US, United States, (2)Plants and Invertebrates, Eurofins Agrosience Services US

With the growing landscape of biostimulants and biopesticides, there is an industry need for the robust quantitation of nucleic acids in environmental fate studies. Environmental fate testing guidelines OECD 307 and OECD 308 are routine tests for the registration of test substances. These guidelines were built on the principles of conventional organic chemistry, where radio-labeled test substances are frequently utilized for ease of analysis. The amount of a substance in soil, sediment, or water can be easily tracked so that the transformation rate, and the distribution of the chemical in water vs. sediment can be calculated over time. However, it is not always practical to use radio-labeling methods when dealing with nucleic acid test substances. Instead, quantitative Polymerase Chain Reaction (qPCR) can be used as an alternative method to measure the change in the concentration of nucleic acids over time. It is important to consider the limitations of this methodology and how it may affect the data being considered for risk assessment. The following are some of the areas for consideration when designing a method validation to support environmental fate studies: extraction method and limits, distribution of test substance in matrices based upon the determined limits, interactions between the test substance and matrix, qPCR primer design and optimization and standard curve creation. Overall, qPCR is a valuable tool to determine the amount of nucleic acids in a test system over time; and, with appropriate measures, it can be considered fit-for-purpose to quantify nucleic acids in environmental fate studies.

4.16.P-Mo333 Risk assessment innovation for double-strand RNA (dsRNA) pesticides with *Caenorhabditis elegans* and *Drosophila melanogaster* as ecotoxicological model

Xupeng Yu and Nico van den Brink, Wageningen University and Research (WUR), Netherlands

Current pesticide risk assessment protocols in the EU are primarily designed for chemical pesticides, with limited standardized approaches for low-risk pesticides. To potentially increase the toolbox for low-risk pesticide Environmental Risk Assessment (ERA), the potential of two in vitro whole organism models, *Caenorhabditis elegans* and *Drosophila melanogaster*, was assessed. Activity and development of *C. elegans* were measured using WMicrotracker ONE, and mortality and development of *D. melanogaster* were assessed with a dsRNA feeding assay. Our results showed that clear, concentration-dependent responses to dsRNA were detected in *C. elegans*. Furthermore, *D. melanogaster* showed no detectable effects in the feeding assay under the tested conditions. These findings demonstrate the potential of *C. elegans* as a suitable species for dsRNA pesticide risk assessment, while *D. melanogaster* may still be a potential model for other types of low-risk pesticides, but not dsRNA pesticides.

Track 5: Life Cycle Assessment and Footprinting

5.01 Addressing Sustainability Aspects of Circular Technologies Using New Approaches in LCA

5.01.T-01 Holistic Sustainability Assessment: Developing a Circularity Indicator Tool for Packaging

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Life Cycle Assessment methods present complex results in a digestible format. Circularity indicators aim to represent circularity in a system through quantitative metrics such as break-even points, reuse numbers, or circularity scores or rankings.

However, whilst these measures are useful for the assessment of circularity, they do not give detail on the environmental sustainability of a product or system and are often somewhat simplistic.

Creating a combined method appears to be a good solution. The challenge lies in ensuring simplicity: A combined indicator which requires both LCA and circularity indicators to be calculated separately and presented together serves only to increase the practitioner's workload and overload the user with complex information.

Through the EU Buddiepack project, a Circularity Indicator tool was developed with the aim of assessing the circularity of packaging. The tool aimed to include environmental sustainability, social impact, economic factors, and circularity ratings.

Three overarching categories were developed: Circular flow of resources, value or economy, and sustainable development. Indicators were included within each of the three categories developed. The Circular flow of resources category was ultimately defined by a modified version of the Product Circularity Indicator. The value category was defined by three sub-categories: Overpackaging, Product waste and Material safety. The Sustainable development category was defined by four categories: Environmental Impact, Economic Impact, Social Impact, and a qualitative category on Total litter.

In each subcategory in the value category, either an existing metric was used or modified, or a specific scoring category was developed. For the three quantitative categories in the Sustainable development category, simplified versions of environmental LCA, economic assessment and social impact assessment were used. All subcategory indicators are calculated in the Circularity Indicator tool, which requires minimal user input and shares data between categories. Standard values for unknown data are offered to the user where necessary, and a large number of packaging materials and formats are built into the tool.

The tool aimed to balance detailed and trustworthy results for both circularity and environmental impact. Though opportunities for improvement exist, testing suggests that it has been successful.

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Innovative solutions to over-packaging and single-use plastics, and related microplastic pollution (IA)

5.01.T-02 Opportunities and limits of new LCA approaches in the circular design of chemical and pharmaceutical manufacturing

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Chemicals and pharmaceuticals deliver essential societal functions but drive substantial environmental burdens across global value chains. Life cycle assessment (LCA) is central to greener and more circular design, yet its use is hampered by scarce primary data, confidentiality, and poor coverage of emerging substances. In this work, we explore how new LCA approaches (parametric inventory models, large-scale and LLM-assisted data mining, and regionalized emission data) can both improve and bias decision-making for circular strategies in chemical and pharmaceutical manufacturing. We combine background data from established databases with foreground models built from primary industrial information, process handbooks, and parametric LCIs embedded in supply chains for selected specialty chemicals. Three industrial case studies are analyzed in “conventional” vs “enhanced” LCA variants: solvent recovery in ethylene glycol production for pharmaceutical manufacturing, regionalized raw material sourcing, and historical steam cracker feedstock switches with implications for future biogenic feedstocks. Results show that scaling solvent recycling and reuse delivers far larger climate benefits than incremental process optimizations (e.g. catalyst choice) in mature technologies, and that regionalized LCIs materially change results where local energy mixes and emission controls differ. LLM-assisted data collection accelerates the construction of regionalized LCIs at moderate coverage, but maintaining data quality at higher coverage levels requires hybrid workflows and robust QA, limiting net effort savings. The feedstock case demonstrates that focusing on ethylene alone can hide higher system-wide emissions from on-purpose propylene (PDH), highlighting the risk of burden shifting when evaluating “circular” feedstock changes. Overall, our findings clarify both the opportunities and limits of emerging LCA tools and data, and provide practical guidance for their use in circular design and supplier engagement in the chemical and pharmaceutical sectors.

Disclaimer/Disclosure:

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5.01.P Addressing Sustainability Aspects of Circular Technologies Using New Approaches in LCA

5.01.P-Tu391 Comparative life-cycle and cultural assessment of anaerobic digestion with and without thermal-hydrolysis pretreatment (THP-AD)

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Sludge treatment technologies deliver environmental, economic, and social benefits by reducing sludge volumes, mitigating hazards, and enabling resource recovery. A comprehensive life cycle assessment (LCA) is essential to identify the most suitable option under real-world constraints.

Existing LCAs of anaerobic digestion (AD) and thermal hydrolysis pretreated anaerobic digestion (THP-AD) are limited by inconsistent inventories and sparse comparative evidence across impact categories, especially when linking biochemical performance to environmental outcomes. Moreover, results are rarely stress-tested across the three ReCiPe perspectives (individualist, hierarchist, egalitarian), and only a few studies explore this dimension alongside environmental results, reducing decision-usefulness.

This study presents a comparative LCA of AD versus THP-AD using ReCiPe. The functional unit is 1 ton dry solids of wastewater treatment sludge treated, with a cradle-to-end-of-life boundary including energy recovery and digestate/end-of-life (EoL) handling. Inventories combine peer-reviewed literature and LCI databases; results are complemented by sensitivity and scenario analyses (e.g., energy mixes, recovery efficiencies).

Environmental results are reported under all ReCiPe perspectives; an exploratory assessment screens cultural perspectives for key stakeholder groups.

Results show that THP-AD improves biodegradability and biogas yields, which offset added pretreatment burdens and reduce global warming potential versus AD. Contribution analysis (CA) highlights the decisive role

of EoL routes and energy recovery. Scenario analysis shows strong sensitivity to the electricity mix, with lower impacts under low-carbon grids, exemplified by a ‘greener’ electricity mix in France (lower CO₂ emissions due to the high share of nuclear power) compared to Belgium. The study will report how results vary across ReCiPe perspectives and summarize these insights.

The study identifies conditions under which THP-AD is preferable and parameters that govern that choice (EoL route, energy mix, recovery efficiencies). The findings provide an evidence-based basis for utilities and policymakers and underscore the role of LCA (with perspective/sensitivity analysis) in evaluating sludge treatment options.

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5.01.P-Tu392 Excellence in Excrements: Circular Management System of Manure

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Livestock manure is one of the main sources of greenhouse gas emissions in the agricultural sector and contributes to land pollution and eutrophication of surface waters. To minimise the environmental impact, a sustainable manure management solution is needed that address environmental challenges while enabling manure to be a valuable raw material in a circular system.

We present a holistic approach for upcycling of low-grade biomass by taking advantage of herbivore digestion system and the resulting manure to create high-value products such as biogas, pulp fibres and a fertilizer precursor. This approach generated high biogas yields (399 LN kg⁻¹ VS) while preserving substantial amounts of lignocellulosic fibres. These fibres were then used to produce high-performance nanocellulosic materials, achieving excellent mechanical properties such as tensile strengths of up to 200 MPa. The environmental impact of this manure-valorisation system was evaluated by lifecycle assessment in a cradle-to-gate approach and compared with two already established processes; Kraft pulp production from hardwood chips and biogas production from agricultural waste. A proxy approach was used to upscale the scenario in which manure was used as raw material from laboratory to an industrial scale. In all impact categories using manure showed to have lower environmental impact than using hardwood as raw material. The results demonstrate that a circular manure management system offers a sustainable alternative to traditional manure treatment giving a valorisation possibility to a condemned raw material.

5.01.P-Tu393 Sustainability Life Cycle Assessment of Modular Swappable Battery Systems for Electric Mobility: A PRISMA-Based Analysis

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The rapid expansion of electric mobility has increased the demand for battery systems that are sustainable, adaptable, and resource efficient. Traditional fixed lithium-ion batteries used in electric vehicles face challenges related to long charging times, material intensity, and limited flexibility, prompting growing interest in modular and swappable battery systems. These systems can reduce charging downtime, extend service life through controlled charging, and improve circularity by enabling repair, replacement, and reuse. However, their overall sustainability performance remains insufficiently assessed.

This study aims to examine the environmental, economic, and circularity implications of modular swappable battery systems through a systematic literature review. Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) approach, a structured search was conducted in major scientific databases, followed by multi-stage screening and thematic classification. The review was organized into three focus areas: sustainability and life cycle impacts of batteries in micromobility, eco-design strategies for light electric mobility, and circularity and end-of-life management.

The final selection included studies covering life cycle assessment, cost analysis, and end-of-life strategies. Findings show that environmental impacts are dominated by the manufacturing phase, while operational practices such as charging patterns and maintenance influence overall performance. Modular designs show potential to reduce full-pack replacements and support more efficient repair and reuse pathways. Circular strategies, including recycling and second-life applications, demonstrate additional benefits, although research specifically addressing swappable configurations remains limited.

Overall, modular swappable battery systems offer promising opportunities to enhance sustainability in light electric mobility, yet current assessments lack harmonized methods and integrated environmental, economic, and social perspectives. The results highlight the need for standardized evaluation frameworks and real-world data to better quantify the benefits and guide future development of next-generation battery technologies.

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5.01.P-Tu394 A Systematic Constraint-Based Life Cycle Assessment Framework for Circular Agriculture: Case Study of Agricultural Plastic Waste Management in Taiwan

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Circular agriculture systems are characterized by high variability, context dependence, and operational uncertainty. In Taiwan, fragmented small-scale farming structures further complicate implementation, creating gaps between theoretical environmental benefits and practical feasibility. However, conventional Life Cycle Assessment (LCA) primarily focuses on environmental performance by aligning functional units. This limits its capacity to capture the real-world constraints that shape circular agriculture. Consequently, the assessment outcomes often fail to provide implementable and contextually grounded guidance.

The emerging Constraint-based LCA concept shifts the analytical focus from functional units to the explicit integration of real-world constraints, including physical, policy, operational, and socio-economic limitations. Yet, empirical case studies are needed to establish the feasibility of this approach across diverse contexts. This study aims to develop a systematic Constraint-based LCA framework to address this gap.

The operational framework is validated using a case study of agricultural plastic waste management in Meinong District, Kaohsiung City, Taiwan. It comprises four steps aligned with, and extending, the ISO 14040 structure: (1) goal and scope definition with constraint identification, informed by stakeholder interviews, policy review, and decision-context analysis; (2) life cycle and constraint inventory, combining conventional LCI with the identification and quantification of constraints across six categories (environmental, economic, social, technical, policy, spatial); (3) system boundary reconfiguration, dynamically adjusting boundaries to reflect processes that remain feasible under the identified constraints; and (4) feasibility evaluation, using context-specific indicators through scenario and sensitivity analyses.

The framework reveals constraint interactions often overlooked by conventional LCA. By explicitly quantifying multiple constraints informed by stakeholder insights, it facilitates the identification of currently viable pathways and the boundary conditions (e.g., subsidies, policy support) required to expand feasibility. The assessment results therefore support pragmatic, context-sensitive decision-making in the Meinong case. The study offers the first set of operational guidelines for applying Constraint-based LCA, supporting the development of more actionable and deployable circular agriculture strategies.

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5.01.P-Tu395 Nitrogen Footprint Focusing on Industrial Sector in Japan Based on Inventory Database in IDEA

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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 footprint focusing on industrial sector in Japan using inventory database of IDEA. We developed the nitrogen input and output amount for each process data adjusted each functional unit (ex. kg, m³, and so on). We collected product amount of each industrial product in a year and connected to process data. Finally, we analyzed total nitrogen amount of target year, 2015. We estimated nitrogen footprint of industrial sector in Japan.

5.01.P-Tu396 Life Cycle Assessment of a new Acrylonitrile-Butadiene-Styrene Dissolution Recycling Technology

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Acrylonitrile-butadiene-styrene (ABS) is widely used in consumer electronics housings, automotive interior components, and small appliances. Mechanical recycling of ABS at end-of-life results in ABS with a lower quality than virgin ABS, due to degradation of the butadiene rubber. A new technology is being developed for dissolution recycling of ABS, recovering virgin-quality SAN polymer. The environmental impact of the ABS dissolution recycling process was studied in a prospective Life Cycle Assessment (LCA) following the Product Environmental Footprint (PEF) method. The data for dissolution recycling is for a full scale dissolution plant (20 kta) and is derived from a model based on lab scale processes. A cradle-to-gate analysis of the dissolution process shows a 65% carbon footprint reduction of recycled SAN compared to virgin SAN and even higher reductions on all other environmental impact indicators. A cradle-to-grave analysis of the application of recycled ABS in car panels using the Circular Footprint Formula from the PEF method shows carbon footprint reductions of 19% compared to virgin ABS when assuming disposal at end-of-life to up 39% when assuming dissolution recycling at end-of-life. Hence, dissolution recycling of ABS can result in a considerable reduction of the environmental impact of ABS, while maintaining a virgin-like quality, making it possible to apply the recycled ABS in a wide variety of products. In combination with the possibility to process difficult-to-recycle waste streams, this technology is could be a valuable addition to mechanical recycling of ABS.

5.01.P-Tu397 Implementing Circular Value Chains in the Oil & Gas Industry: A Sustainability Assessment of Crankshaft Remanufacturing

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R3-MYDAS is a Horizon Europe project that aims to accelerate the manufacturing industry's transition toward sustainable and circular value chains. A key aspect of this effort is the implementation of Sustainability by Design (SbD) principles to ensure that innovative circular processes generate environmental, economic, and social benefits. In this context, the project encompasses three industrial demo cases, with a focus here on the oil & gas sector's crankshaft component.

Traditional linear value chains in the oil & gas industry often lead to component disposal when failures occur, generating significant environmental and economic costs. The R3-MYDAS project proposes a remanufacturing approach for crankshafts, enabling repair and reintegration during maintenance and end-of-life. To achieve this, worn areas of the crankshaft are scanned to create a 3D model, guiding the design of the required clad layer,

which is then applied using laser cladding. While this innovative process restores components to near-original specifications, improving precision, cost-efficiency, and environmental performance, understanding its broader impact requires a structured evaluation.

To this end, an SbD assessment has been conducted for the crankshaft demo case, focusing on environmental and socio-economic dimensions to ensure that the remanufacturing strategy is both sustainable and socially responsible:

- **Environmental dimension:** A Life Cycle Assessment (LCA) evaluates the environmental impacts of the remanufacturing process, including carbon footprint and resource efficiency, following ISO 14040 guidelines. The assessment highlights both the potential benefits of shifting from a linear to a circular value chain and the environmental hotspots where further improvements could enhance sustainability.
- **Socio-economic dimension:** Life cycle cost analyses are conducted to ensure that the remanufacturing approach is economically viable and competitive with conventional production and maintenance methods. In addition, the assessment also considers potential social implications across the value chain, such as workforce adaptation and occupational safety.

The proposed remanufacturing strategy is designed to be sustainable, with potential for further optimization to improve process efficiency and support wider industry adoption. The crankshaft demo case illustrates how circular strategies can be implemented in industrial contexts, showing how SbD can guide circular strategies.

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5.01.P-Tu398 Eco-Design and Carbon Footprint Minimization in 3D-Printed Drug Products through Integrated Production Efficiency and Sustainable Logistics

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The rapid expansion of three-dimensional (3D) printing in pharmaceutical manufacturing offers a transformative pathway toward personalized medicine, on-demand production, and improved therapeutic precision. However, the environmental implications of 3D-printed drug products remain insufficiently explored, particularly with respect to material use, energy demand, and distribution-related emissions. This project addresses these challenges by investigating how eco-design principles and carbon footprint minimization strategies can be integrated across the full value chain of 3D-printed pharmaceuticals.

The study focuses on the development of a sustainability-oriented framework that combines product eco-design, production efficiency, and sustainable logistics to reduce the environmental burden of drug manufacturing. At the product level, eco-design approaches are applied to optimize formulation composition, minimize excipient and packaging waste, and improve printability while maintaining drug quality, dosage accuracy, and patient-specific functionality. At the process level, the project evaluates critical manufacturing parameters affecting energy consumption, material efficiency, and production yield, aiming to identify operating conditions that lower emissions without compromising performance or regulatory compliance.

Beyond production, the project examines the logistics dimension of decentralized and on-demand pharmaceutical manufacturing. By comparing centralized and distributed supply models, it assesses the potential of localized 3D printing to reduce transportation distances, storage requirements, and associated greenhouse gas emissions. Life Cycle Assessment (LCA) and carbon footprint analysis are used as core tools to quantify environmental impacts and identify hotspots throughout raw material sourcing, printing, post-processing, packaging, and delivery stages.

The expected outcome is a set of practical design and operational guidelines for more sustainable 3D-printed drug products, supporting the transition toward greener pharmaceutical systems. By linking engineering optimization with environmental assessment and supply chain innovation, the project contributes to the broader goals of circular economy, low-carbon healthcare, and resilient medicine production. Ultimately, it demonstrates how 3D printing can evolve from a technological innovation into a sustainability-enabling solution for the future of pharmaceuticals.

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5.01.P-Tu399 Do Rental and Sharing Models Deliver? Life Cycle Assessment-Based Lessons on Logistics, Use Intensity and Circular Design

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Rental and sharing systems are increasingly promoted as circular business models with the potential to reduce environmental burdens by replacing individual ownership with collective use and preventing underutilization of products. However, their actual sustainability performance depends strongly on logistics, use patterns, and system design. Within a local Green Deal project on Rental and Sharing in Flanders (Belgium), a streamlined Life Cycle Assessment (LCA) focusing on climate change and resource use was performed on 7 case studies. The case studies were selected to reflect variation in product types, target customers (B2C vs B2B), organizational scale, seasonality in use, use cycle duration, and geographical coverage of the service. The objective was to identify the conditions under which renting and/or sharing delivers net environmental benefits compared to traditional purchase models, and to derive general recommendations for implementing rental and sharing systems. Each rental or sharing model was compared with its corresponding reference scenario, typically a traditional purchase scenario. The evaluation followed LCA principles, accounting for environmental impacts across the full life cycle of a product: production, distribution, use, repair activities, and end-of-life processing. All transportation steps associated with use and repair activities are considered. This encompasses transport to the consumer and back to the store, as well as transport to and from repair facilities, where applicable. Results indicate that products with relatively high production impacts and low use rates are particularly suitable for shared use, and repairable product designs and logistics emerged as critical drivers of environmental performance. Rental and sharing models can offer substantial benefits in terms of resource efficiency, as fewer products are required to meet the total user demand. However, the implications for climate change are more complex. When products are rented or shared at high frequency and for short usage periods, customer transport to and from the pick-up location - particularly when conducted by car - can offset or even exceed the climate benefits achieved through reduced production volumes. To ensure that environmental gains are effectively realized, it is therefore crucial to optimize transport logistics, for example by promoting alternative, low-carbon modes of mobility or by organizing the rental or sharing service as locally as possible.

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5.01.P-Tu400 Waste Footprint Analysis in Japan Using Waste Input-Output Model for Advancing Waste Circularity

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The transition to a circular economy requires robust methods to quantify waste flows induced by consumption across complex supply chains. This study quantifies Japan's consumption-based waste footprint in 2020 using an extended Waste Input-Output (WIO) model. The WIO framework integrates waste generation, treatment, and recycling flows into conventional input-output analysis, enabling systematic tracking of waste directly and indirectly induced by final consumption both domestically and abroad.

Traditional waste accounting often overlooks indirect waste embedded in supply chains, limiting policy insights. To address this, we constructed a 2020 WIO table by harmonizing Japan's official Input-Output Tables and industrial waste statistics. The resulting model integrates production sectors and waste management sectors—such as recycling, landfill, and incineration—within a unified matrix framework.

Using this model, we estimate Japan's total waste footprint as approximately 635 million tons, with 334 million tons generated domestically and 301 million tons abroad. Major waste generation occurs in food manufacturing, construction, and service sectors, underscoring the importance of supply chain perspectives. Domestic waste treatment relies heavily on incineration, raising concerns about greenhouse gas emissions, while recycling predominantly serves the cement and agricultural industries, indicating sector-specific circular flows.

The study's single-country scope limits detailed tracing of foreign waste destinations, highlighting the need for future multi-regional extensions to better capture global waste flows. Nonetheless, the WIO model offers a powerful tool for comprehensive waste flow assessment and supports evidence-based policies for circular economy transitions.

This work provides critical insights into consumption-induced waste dynamics and treatment pathways in Japan, contributing to sustainable waste management strategies and advancing methodologies for global applications.

5.01.P-Tu401 The potential of calcium and magnesium silicate as a circular input for correcting soil acidity and decarbonizing maize production in Brazil.

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The reuse of inputs from one production chain in another supports the circular economy and contributes to decarbonization. Calcium magnesium silicate (CMS), obtained from stainless steel slag, can act as a soil acidity corrective, partially or fully replacing calcitic or dolomitic limestone. Although technically equivalent, it is necessary to assess whether metals present in CMS (e.g., chromium, lead, cadmium) may generate adverse effects on human health and ecosystems. This study evaluates the effects of metal bioaccumulation from CMS applied in maize cultivation in Mato Grosso, Brazil. The environmental profile of CMS was analyzed using life cycle assessment (LCA), considering its application in soil for the production of 1 ton of maize grains at 13% moisture. System boundaries cover stages from planting to harvest, including fertilizers, pesticides, and machinery fuel. We defined 3 scenarios: S1 (100% limestone), S2 (50% limestone and 50% CMS), and S3 (100% CMS). Impact categories include climate change (IPCC 2021 - GWP 100y), acidification, and freshwater ecotoxicity and eutrophication (Environmental Footprint 3.1), and human toxicity - cancer and non-cancer (USEtox 2). Calculations were performed in SimaPro v.9.6 with ecoinvent v.3.10. Climate change impacts decrease from 260.63 kg CO₂ eq in S1 to 241.07 kg CO₂ eq in S2 and 221.52 kg CO₂ eq in S3 (15% reduction). Acidification remains nearly constant (7.68 mol H⁺ eq), varying only 0.19%. Terrestrial eutrophication changes from 33.98 to 33.95 mol N eq (-0.09%). Freshwater ecotoxicity rises from 6452.67 to 7293.21 CTUe (13%). Freshwater eutrophication falls from 0.0288 to 0.0278 kg P eq (3.5%). Human toxicity-cancer decreases from 2.06E-07 to 2.03E-07 (1.08%), while human toxicity-non-cancer increases from 2.88E-07 to 2.90E-07 (0.70%). CMS, even within regulatory limits, can increase human toxicity and freshwater ecotoxicity if applied excessively or repeatedly. Results also indicate significant differences in CO₂ between CMS and limestone, as CMS does not undergo carbonation in soil. Differences in other impacts are minor and may reflect LCA uncertainties. Overall, CMS shows environmental performance equivalent to limestone. Moreover, its reuse replaces conventional inputs without performance losses and mitigates impacts associated with the underutilization of this metallurgical by-product, contributing to more sustainable material management and a circular economy.

5.01.P-Tu402 Synergizing Life Cycle Assessment and Logistics to Improve Supply Chain Management

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Reusable packaging is increasingly promoted as a strategy to reduce waste and environmental impacts, yet its actual performance depends on complex interactions between product design and supply-chain logistics. This study demonstrates how combining Life Cycle Assessment (LCA) with logistics analysis strengthens supply-chain decision-making, drawing on insights from the BUDDIE-PACK project across four use cases: takeaway food containers, refillable detergent bottles, semi-rigid catering trays, and on-the-spot consumption containers. Using cradle-to-grave LCA aligned with Product Environmental Footprint (PEF) principles, the analysis quantifies impacts across production, transportation, washing, use, and end-of-life stages. Key environmental hotspots are identified in material production, washing operations, transport distances, and disposal pathways. Break-even analyses and sensitivity tests further examine how factors such as washing efficiency, consumer transport, and packaging mass influence the environmental viability of reuse.

The results show that reusable solutions only outperform single-use options when supported by efficient and well-designed logistics loops. High-efficiency washing, minimized transport burdens, lightweight yet durable designs, and credible recycling routes meaningfully reduce environmental impacts.

Overall, integrating LCA with logistics provides a realistic and actionable roadmap for scaling reusable packaging systems. The synergy provides a robust foundation for designing supply chains that are both environmentally resilient and operationally viable.

5.01.P-Tu403 Exploring the Use on Land Phase in Nutrient Recovery: Nuancing the Fertilizer Substitution Principle in Life Cycle Modelling with tier 3 model Daisy

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Nutrient recovery (NR) technologies in wastewater treatment (WWT) are increasingly promoted as circularity solutions, yet life cycle assessment (LCA) studies often use simplified fertilizer substitution and use on land (UoL) approaches that overlook site-specific agronomic dynamics. While previous research shows that methodological choices around fertilizer substitution strongly influence LCA results, detailed UoL modelling remains rare. This study evaluates how integrating the tier 3 agro-ecosystem model Daisy within a consequential LCA framework can refine assessments of bio-based fertilizers (BBFs) from NR systems.

The UoL stage of an ammonium-rich BBF produced at a pilot scale ion exchange/adsorption unit integrated into municipal WWT was modelled using Daisy. Simulations were parameterized with site-specific soil, weather, crop rotation, and management data from a three-year field trial, supplemented with BBF mineralization data from pot experiments and all scaled to the same yield/area standard. Three BBF application scenarios were modelled: (1) BBF with a measured N content of 4 kg N/ton BBF, and alternative optimized scenarios using (2) natural zeolite with a higher adsorption capacity and (3) polymeric resin. A reference scenario with only mineral NPK fertilizer application was also modelled. Results were translated into consequential LCA models and compared to scenarios where tier 1 IPCC emission factors were used with the same 3 BBF scenarios modelled in Daisy.

Results between the 6 modelled scenarios varied only slightly. In the climate change category, an impact reduction of 7.05 and 2.2% was observed in Daisy modelling compared to IPCC UoL results in scenarios using zeolite with an adsorption capacity of 4 kg N/ton and resin with 100 kg N/ton, respectively. The small change in impacts is explained by similar properties of the BBF and synthetic fertilizer. To support LCA practitioners in tool selection in the UoL phase, a decision aid was developed based on key factors such as decision context, UoL impact share, BBF type and mineralisation behavior, novelty and type of BBF. Model calibration with measured data is recommended to ensure robustness.

Overall, integrating Daisy into NR LCAs enhances representativeness and provides a stronger basis for evaluating BBFs and NR technologies within circular nutrient management. The proposed decision aid contributes towards more consistent and transparent UoL modelling practices.

5.01.P-Tu404 Advancing Circular Life Cycle Assessment Through Time-Explicit Modelling and System Expansion. A Case Study for Wind Turbine Systems

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Transitioning to a renewable energy system is rapidly increasing the demand for Critical Raw Materials. Recovering these critical materials through circular practices can be a great opportunity for the European Union. Traditional LCA is well suited for evaluating linear product systems. However, the application of conventional LCA to circular systems raises several methodological challenges, most notably those related to multifunctionality and to the allocation of burdens and credits associated with end-of-life recycling processes. Combining system expansion to model multiple lifecycles of a given product system with time-explicit LCA could solve these issues.

In this study we model and compare two different wind turbine product systems in Belgium with 1 kWh of electricity as functional unit. Both product systems have two lifetimes of wind turbines. In the first product system, referred to as the linear system, both the initial wind turbine and the second wind turbine is manufactured from virgin materials. It has standard, non circular end-of-life strategies at its end of life. In the second product system, referred to as the circular system, the initial wind turbine is likewise manufactured from virgin material. The second life of the wind turbine is modelled from recycled materials recovered from the first wind turbine.

The initial results show that applying circular practices can significantly lower global warming impacts. The extra environmental burdens added from the recycling processes are smaller compared to the environmental impacts of manufacturing a second wind turbine from virgin materials. In essence, recycling results in lower environmental impacts than extracting and processing the raw materials from their ores.

Integrating a temporal dimensions into the model is still a work in progress. We aim to achieve this by conducting a time explicit LCA. It is expected that both the linear as the circular product system will have lower environmental impacts in their second life due to the decarbonization of the economy. How this temporal dynamic will eventually influence the final results is yet to be determined at the time of writing. The goal of this work is to further develop methodologies related to circular LCAs by combining time explicit LCA with system expansion. This could potentially solve certain issues conducting LCAs of circular product systems and improve the level of detail of these LCA studies in general.

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5.01.P-Tu405 Assessing the risk of Pharmaceuticals in the Environment (PiE) as a result of recycling primary packaging

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Pharmaceutical packaging take-back and kerbside recycling schemes are increasing due to industry commitment to circular economy principles. In the UK, some high street pharmacies offer medicine blister recycling, while Swedish patients can recycle empty pharmaceutical packages in household bins. There is however a lack of published research on the environmental risk posed by the release of active pharmaceutical ingredients (APIs) during recycling or on the typical levels of API residue on packaging materials after use. API residues left on packaging may enter rinse water at recycling centres and, depending on wastewater treatment, could be discharged into the environment, presenting a potential localised pollution risk.

This case study uses AstraZeneca's Safe API Discharge Programme to assess Pharmaceuticals in the Environment (PiE) risks from recycling blister packs. The programme calculates a Risk Quotient (RQ) by comparing theoretical discharge quantities to a Predicted No Effect Concentration (PNEC), usually used to assess manufacturing emissions. In this study, residue values documented for coated tablets stored in HDPE bottles were utilized as conservative estimates due to the lack of blister-specific data. Three hypothetical APIs with varying ecotoxicity were assessed across different recycling volumes, assuming full compliance and daily processing at a single facility, with standard effluent discharge and dilution factors. Results indicate that for APIs with medium and high PNECs, PiE risk from blister pack recycling is negligible. However, recycling moderate to high volumes of blisters containing highly ecotoxic APIs may result in a risk ($RQ > 1$) under certain circumstances. This underscores that a universal risk assessment is not feasible, as API ecotoxicity and recycling volume both influence PiE risk. Scheme type also matters as takeback schemes typically process smaller volumes at a single site, while kerbside recycling may result in larger, decentralised volumes. We explore other contributing factors to recycling volumes and also consider the impact of dose form (blister, inhaler or injection pen) on the outcome of the assessment. The assessment demonstrates the importance of tailoring risk evaluations – to the API, packaging type, local conditions, and scheme methodology – and refining assessments as recycling programmes expand. Further work may include analysis to verify API residues on blisters to support the desktop assessment.

5.02 Advancing Methods for Prospective Life Cycle Assessment to Support Radical Approaches to Sustainable Research and Innovation

5.02.T-01 Dynamic Counterfactual LCA: Prospective Consequences via Socio-ecological Modeling

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Beyond its standard description as a life-cycle modeling approach, LCA actually consists of two distinct logics: attributional LCA, which assigns average environmental burdens to products themselves, and consequential LCA (CLCA), which propagates effects of decisions through production systems and markets. In practice, CLCA relies on simplified heuristics that capture only short-term or proximate effects, whereas real-world decisions can trigger delayed, amplifying, or nonlinear consequences.

This work introduces Dynamic Counterfactual LCA (DC-LCA), an extended form of CLCA designed to capture

such long-term, system-level propagation effects. Rather than setting the studied product system in the future as in traditional Prospective LCA, DC-LCA models a present-day system while projecting consequences dynamically into the future. It integrates System Dynamics (SD) modeling to explicitly represent delays, feedbacks, and the evolution of social and environmental variables over time. By simulating a counterfactual world evolving without the studied intervention associated with the functional unit, impacts can be derived from the dynamic integration of differences between “with” and “without” scenarios at each point in time.

We demonstrate the approach using a socio-ecological SD model representing the spread of vegan diets within a population. A single individual switching diet for one year is introduced as a perturbation to the model associated with a functional unit, creating small but time-dependent deviations in vegan and omnivore population trajectories. These delayed deviations are translated into temporally resolved demand changes, enabling total environmental consequences to be computed using a dynamic and prospective LCA framework.

Results show that delayed, system-mediated effects dominate the immediate impact of the dietary change. Impacts are nonlinear and non-symmetric: reversing the dietary change or scaling its magnitude does not simply invert or proportionally adjust total emissions.

DC-LCA thus departs from the linear assumptions of classical CLCA by embedding decisions within evolving social and environmental systems. It offers a more realistic representation of causality, the sole necessity for the canonical definition of CLCA, highlighting that the consequences of actions depend not only on current supply chains but also on the social dynamic context in which they unfold.

5.02.T-02 Prospective Life Cycle Assessment in Mineral Value Chains: an Extend SIMPL Approach (SIMPL-Minerals)

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As the energy transition accelerates, the need for minerals is expected to rise sharply, bringing increased attention to the environmental consequences of expanding their supply. Prospective Life Cycle Assessment (pLCA) offers a way to estimate these future impacts. Yet many pLCA studies in the mineral sector rely on different assumptions and parameters, making their results difficult to compare. To support more consistent prospective life cycle inventory (LCI) modelling in this context, this study introduces a structured approach. Our approach builds on the existing SIMPL framework for scenario-based pLCA modelling and extends it with elements tailored specifically to mineral value chains, forming the SIMPL-Minerals (SIMPL-M) approach. These additions include defining the key stages and processes of mineral supply chains, identifying the parameters linked to those stages, and connecting these parameters to broader macro-level drivers that shape future developments. The resulting parameter set was reviewed and refined through semi-structured expert interviews. We then applied the SIMPL-M approach in an exemplary case study assessing future global nickel sulfate production for batteries.

Using SIMPL-M, we identify 18 central parameters that should be considered when conducting pLCA of mineral supply chains. These parameters reflect variations in deposit characteristics, technologies, and geographic conditions, and span both primary and secondary production, from ore extraction or waste collection to refined materials. Each parameter is positioned within a generic inventory model blueprint to show where it becomes relevant along the value chain. By linking these parameters to macro-level factors, the approach supports coherent scenario construction and consistent assumptions about how parameters evolve over time. When applied to the nickel sulfate case study, SIMPL-M demonstrates that incorporating parameters beyond conventional background LCI data can meaningfully alter impact assessment outcomes.

By adapting the SIMPL framework to the specific needs of mineral value chains, we develop the SIMPL-M approach. SIMPL-M supports practitioners in applying parameters systematically within inventory modelling and constructing scenarios grounded in macro-level drivers influencing parameter change. In doing so, it improves comparability across pLCA studies and reduces modelling effort for users.

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5.02.T-03 Climate change impacts with prospective characterization factors: sensitivity of background inventories

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Prospective life cycle assessment (pLCA) is increasingly used to evaluate future environmental impacts as technologies, socio-economics, and policy contexts evolve. While methodological advances have strengthened prospective life cycle inventory modelling, progress in prospective life cycle impact assessment has remained limited. Recent work addressed this gap by developing prospective characterization factors (pCFs) for climate change, consistent with IPCC methods and derived from Integrated Assessment Model (IAMs) scenarios under different Representative Concentration Pathways (RCP) and Shared Socioeconomic Pathways (SSPs). These pCFs capture shifts in radiative efficiencies and impulse response functions for CO₂, CH₄, and N₂O, revealing substantial variation in future climate metric values and enabling their integration into LCA tools. Building on this foundation, the present study applies the new pCFs in a comprehensive sensitivity analysis of all activities inecoinvent. Using Brightway2 and IAM-RCP-SSP specific GWP100 and GTP100 values for 2030, 2040, and 2050, we examine how emission timing, scenario choice, and climate metric selection influence future climate impacts. Results show that activities dominated by non-CO₂ gases are most sensitive to pCFs, with methane- and nitrous-oxide-intensive systems exhibiting distinct response patterns, while fossil-CO₂-dominated activities remain comparatively stable. These findings highlight where prospective modelling most affects outcomes, support better interpretation of long-term uncertainty, and strengthen the coherence and decision relevance of prospective life cycle impact assessment.

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5.02.T-04 Is Time-Explicit Life-Cycle Assessment Useful? A Vehicle Eco-design Case Study

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Life-cycle assessment (LCA) is increasingly used for eco-design. Time-explicit LCA is emerging as a framework for the coherent inclusion of temporal dynamics. However, for the additional efforts of this approach to be justified, it should improve the value of the assessment to its application. The question is therefore whether time-explicit LCA can yield insights beyond those obtained with conventional (temporally static, near-present) LCA or prospective (temporally static, future) LCA. To answer this question, we consider a case study in automotive eco-design: the development of a polypropylene (PP) composite with a softwood filler, which could substitute talcum-PP in the production of interior trims. Both alternatives are assessed from cradle to grave, considering components produced from 2030 to 2055.

Moving from a system based on primary material, using considerable fossil resources (near-present static), to a circular system using more renewable energy (prospective static) consistently leads to impact reduction. Following life-cycle stage contribution analysis, the temporal perspective influences which activities are identified for potential impact mitigation. Softwood-PP enables lightweighting and has a lower polypropylene volume, generally making it the preferred alternative in temporally static perspectives. However, with the assumption that increased circularity is primarily driven by the mechanical recycling of secondary compound, talcum-PP is at a distinct advantage, as a future feedstock of secondary softwood-PP relies on this novel compound being introduced in the first place. Consequently, a time-explicit perspective indicates a preference for talcum-PP.

Based on these results, it becomes a priority to investigate whether a softwood filler could be combined with secondary talcum-PP – either through chemical recycling or by creating a hybrid composite – which could bring the preference of a time-explicit perspective in line with the temporally static results. The relevance of these questions is not apparent from either temporally static perspective. Considering the system as temporally differentiated leads to a more holistic assessment, which can indeed yield novel insights. This case study highlights the value of time-explicit LCA, especially concerning design for a circular economy. While the practical application of time-explicit LCA still faces many challenges, our results indicate that these challenges are worth addressing.

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5.02.P Advancing Methods for Prospective Life Cycle Assessment to Support Radical Approaches to Sustainable Research and Innovation

5.02.P-Tu406 Structuring the Application of Life Cycle Assessment to Emerging Technologies: A Multi-Perspective Approach

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Modelling decisions made during the goal and scope definition stage can substantially influence LCA outcomes and might result in model-dependent conclusions. This issue becomes even more pronounced when assessing emerging technologies, characterised by high design freedom. In such situations, LCA faces additional challenges, including limited comparability, data scarcity, and/or high uncertainty. In this context, we propose a novel, structured approach to applying LCA that models emerging technologies from multiple perspectives depending on the technology readiness levels.

Based on the inductive reasoning of 20+ publications of the first and second author on LCAs of emerging technologies, a set of research questions were formulated that aligns with the technologies maturity level. Additionally, the reasoning was challenged, criticed and scrutinized by three invited experts from the LCA community who joined the work for this purpose and have proposed revisions to increase rigor.

The resulting multi-perspective approach structures the application of LCA to emerging technologies by aligning goal and scope definitions with the technology's maturity, from conceptual development to full-scale implementation. The six perspectives are: theoretical assessment (TRL 1–2), laboratory scale (TRL 3–5), industrial scale-up (TRL 4–6), lifecycle integration (TRL 6–9), market consequences (TRL 6–9), and future estimations and target benchmarking (TRL 3–9). Recommendations are given for each perspective concerning the LCA goal, system boundary, functional unit, temporal and geographical scope, and applicability of conclusions.

Starting point for the present work is the hypothesis that LCA conclusions are inherently model-dependent, substantially shaped by modelling decisions made during the goal and scope definition stage, an issue that becomes particularly significant when assessing emerging technologies. As a possible way to overcome this, a multi-perspective approach that privileges explorative LCA modes is proposed and aligned with the TRL levels, providing structure to the application of LCA during technological development.

5.02.P-Tu407 Future Analysis of Environmental Impacts by Combining Japan's Energy System Model With LCA

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Since the adoption of the Paris Agreement, ambitious greenhouse gas emission targets have been set in many countries. In Japan, increasing attention has been directed toward more stringent future environmental roadmaps, such as the Carbon Neutral 2050 target. Furthermore, interest in environmental impact categories beyond climate change is growing, leading to an increase in studies incorporating multi-criteria analysis, including biodiversity, resource consumption etc.

Along with the growing awareness of environmental issues, diverse methodologies for assessing environmental impacts have been developed. Energy system models (ESMs) are computer programs that organize the components of an energy system, depicting the flow from energy production, transport, and conversion to consumption. The TIMES-Japan, developed by the Institute of Applied Energy (IAE), is an ESM used to determine Japan's future electricity generation mix and technology deployment, and is employed in environmental policy making and evaluation. Life Cycle Assessment (LCA) is a widely used methodology for quantifying diverse environmental impacts of products and services.

While both systems assess environmental impacts, each has its own challenges and constraints. ESMs tend to focus on CO₂ emissions and cannot evaluate environmental impacts other than climate change. On the other

hand, in LCA, the validity of the results depends on the degree of data availability, and a key challenge is the lack of background data when evaluating future technologies.

In previous studies that attempted to integrate ESMs and LCA, challenges such as database consistency and model-induced uncertainties have been identified. This study aims to quantify Japan's future environmental impacts while addressing these challenges.

In this study, the Premise framework is employed to couple the Ecoinvent with TIMES-Japan, thereby creating an inventory database that reflects a future energy system structured in accordance with Japan's environmental roadmap. The resulting database will be validated by comparison with other regional and global-scale case studies, and a framework will be developed to incorporate diverse social and technological scenarios.

The application of the developed framework contributes to the creation of background data for Prospective LCA, as well as to the incorporation of multifaceted environmental impacts into ESMs that have traditionally relied solely on CO₂ emissions as constraints.

5.02.P-Tu408 Sea vs Land Mining for Manganese Products: Comparative Prospective Life Cycle Assessment

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The transition to renewable energy will entail an increased demand for materials, but the future supply of many of the required materials is sensitive to geopolitical and trade factors. This is especially true for Europe, as many of these materials, including manganese, are principally mined overseas. One emerging technology that could provide an alternative source of materials is sea mining of polymetallic nodules, either from the deep sea or shallower waters (~100 m). However, little is known about its environmental impact. This has sparked a global debate, and several countries have called for a moratorium on sea mining until more is known about its environmental impact. To date, few studies have quantified the environmental impacts of sea mining in direct comparison with land-based mining, and no studies focus specifically on sea mining in European waters.

In this initiated study, we provide insight into the environmental impacts of sea mining in European waters by performing a prospective life cycle assessment of sea mining in an area of manganese-rich polymetallic nodules located in the shallow waters of the Bothnian Bay (~80 m). These impacts are also compared to those of “conventional”, land-based mining of manganese. To perform the assessment, data is provided by a collaborating sea mining company called Scandinavian Ocean Minerals. The aim of this study is to investigate which of the following options has the lowest environmental impact from a life cycle perspective: (1) manganese materials from nodules mined in the Bothnian Bay, or (2) manganese materials from ore mined on land.

The environmental impacts will be quantified for two manganese reference products important to the green transition and the current industry: 1 kg manganese sulfate used in battery production (e.g., in nickel-manganese-cobalt [NMC] lithium-ion batteries), and 1 kg ferromanganese used as an alloy in steel production. Main results will be presented for the impact categories global warming and mineral resources. We will also address biodiversity impacts using various biodiversity indicators. Considering the immature state of sea mining, a prospective inventory modeling is performed for the years 2030-2050. Background system data are adjusted over time according to different future scenarios.

With the results of this study, we hope to contribute with quantitative data and comparative perspectives to the growing global debate on the environmental impacts of sea mining.

5.02.P-Tu409 Coupling LCA and Stock / Flow Model for the Assessment of Concrete Waste Management Future Scenarios

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With about 6 billion cubic meter per year, concrete is the most widely used construction material at global scale. Because of its cement content, concrete manufacturing is responsible for a major part of the embodied greenhouse gases (GHG) of buildings and civil engineering infrastructure. Concrete manufacturing also leads to the extraction and use of primary resources, such as sand and aggregates, and to the generation of large flows of construction and demolition concrete waste. This study aims at assessing future concrete end-of-life strategies at

national level through the combination of MFA and LCA. Based on a functional unit defined as “1 management of the concrete waste volume generated in France during a 10 year period starting from 2025”, it explores scenarios incorporating sub-scenarios of waste outflow generation (business as usual BAU, less concrete, low demand, longer lifetime, recycling), as well as sub-scenarios of concrete end-of-life management (landfilling, backfilling, reuse, recycling in road and concrete, recycling in concrete with accelerated carbonation). The considered time horizon is 2025 to 2100 with a 10 year time step. The methodological approach consists in coupling Material Flow Analysis (MFA) with Life Cycle Assessment (LCA) through the integration of two MFA sub-models (inflow-based + stock-based) in the Flexibe Open Dynamic Material Systems Model (flodym), linked with supply chain graph-based LCA model developed in Brightway 2.5. Results show that scenarios maximizing the recycling and accelerated carbonation of recycled concrete aggregates might lead to a reduction of impacts on climate change due to CO₂ carbon capture. This environmental improvement is however counterbalanced by an increase in other environmental impact categories due to increase consumption in electricity for accelerated carbonation processes.

Disclaimer/Disclosure:

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5.02.P-Tu410 Life Cycle Environmental Impacts of Digital Services: Mitigation Strategies for Video Streaming

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The information and communication technologies (ICT) sector is responsible for large shares of energy and material consumption, contrary to common and misleading perceptions of digital ‘dematerialization’ or of the largely invisible ‘cloud’. End-user devices, networks, and data centers are very material parts of digital services supply chains. With an increasing share of global digitalization, the consequences of ICT are expected to grow in the future. Indeed, ICT is the cause of 5 to 7% of global electricity consumption, a share that is forecasted to double by 2030. ICT is also increasingly dependent on key minerals and metals, and is a primary driver of critical raw materials such as indium, gallium, and germanium. Robust models to quantify the environmental impacts of digital services’ supply chains are urgently needed.

This study presents a prospective life cycle assessment (LCA) of ICT focused on the digital service of video streaming and its supply chain. We modelled the life cycle environmental impacts of one hour of video-on-demand streaming to an LCD TV in the EU, including interventions such as substituting components in TVs, and alternative hardware in data centers. Results provide insights into the contribution of the main stages of the supply chain as well as into the mitigation potential of the interventions considered.

This work illustrates the ability of prospective LCA to provide insights into digital services and associated explorative mitigation strategies. It highlights the need for models that (1) account for the entire life cycle of digital services, and (2) include impacts beyond climate change. Insights gained following this approach lead to recommendations that support individuals, technological innovators, and policymakers; three actors who all have a role to play in advancing a resilient and sustainable ICT sector.

5.02.P-Tu411 Towards robust and consistent, yet technology-specific prospective life cycle assessment

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As a technology develops from idea to production, we gather information and thereby reduce the uncertainty when estimating the industrial-scale environmental footprint. We have a growing knowledge of our new technology, but at the expense of influence. At the time that we know a lot, we have little influence left, so it becomes difficult to decrease the footprints. At an early stage, we have more influence, but little information. Prospective Life Cycle Assessment (pLCA) allows an emerging technology, still in early development, to be modeled at a future, more-developed phase. The goal of our work is to practically demonstrate a comprehensive and generalizable approach to perform technology-specific pLCA, which can lead to realistic environmental impact predictions on industrial scale.

For this, we applied a three-phase pLCA framework to case studies on varying technologies. Each technology had a current TRL between 5 and 7 and was upscaled to TRL 9, including scale-up changes, and sometimes

industrial learning and/or external developments. Further, methods were developed to estimate industrial scale footprints for pharmaceutical synthesis from industrial scale data. And a method was developed to assess process-specific learning and include external developments.

We showed that the relative importance of each technological development to the future environmental footprint of a new technology can differ between product systems. Further, including a range of impact categories is important to prevent burden shifting and to focus on minimizing overall environmental impact. Finally, technology preference can change in the future due to developments in the background systems, leading to quantitative changes in the impacts of the foreground system, but also to an altered avoided burden. Our new methods to quantify impacts of upscaling technologies showed that Process-specific learning curves in prospective LCA provide practical solutions for integration of learning in foreground and background processes of new technologies produced at industrial scale. Moreover, they enable contribution analyses and the separate assessment of developments in the foreground and background system. More process-specific learning curves need to be developed to ensure robust upscaling. Our developed framework and methods contribute to more certain predictions, ensuring early-phase influence to minimize environmental impacts of new technologies.

5.02.P-Tu412 Prospective Macro-Level Life Cycle Assessment: A Systematic Review

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Transitions to sustainable societies require assessments of future environmental impacts at the macro-level. We examined how prospective process-based Life Cycle Assessment (LCA) is used to model environmental impacts at national to global scales. Our research objectives were to (i) provide an overview of modelling approaches in prospective macro-level LCA; (ii) identify common pitfalls and best practices; and (iii) highlight key challenges and suggest priorities for future research.

We reviewed 86 peer-reviewed studies, selected from an initial set of 925 articles, with a primary focus on how system scaling, temporal evolution, and temporal distribution were addressed in the inventory analysis phase. In addition, we assessed elements from the other three LCA phases, including research objectives, temporal scope, system boundaries, and the treatment of sensitivity and uncertainty. We also examined terminology use and transparency.

We classified the reviewed approaches by how system scaling is treated in the foreground system: (i) coupling with Dynamic Stock Models, which captures stock dynamics but overlooks socio-economic aspects; (ii) coupling with Energy System Models, which provides detailed insights but is limited to the energy sector; (iii) coupling with Integrated Assessment Models, which offers broader socio-economic coverage but operates at coarse resolution and typically requires collaboration with model developers; and (iv) uncoupled approaches, which allow flexibility but risk oversimplification. We identified twelve key pitfalls, including simplified treatments of system scaling, temporal dynamics, and distribution; a narrow climate focus; limited scenario diversity; and weak internal consistency. We also highlight best practices for avoiding these pitfalls.

Our review reveals a diverse field with inconsistent terminology, assumptions, and modelling practices. To strengthen the field, we recommend improving transparency and adopting consistent terminology; improving the representation of the complexity of sustainability transitions; strengthening policy relevance; and developing methodological guidance. Addressing these priorities will improve the robustness of prospective macro-level LCA and advance our understanding of sustainability transitions.

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5.02.P-Tu413 Toward a Generalized Scale-Up Framework in Prospective Life Cycle Assessment

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Prospective Life Cycle Assessment (pLCA) plays a crucial role in evaluating emerging technologies from the early stages of development. A major challenge is the absence of generalized approaches for translating laboratory-scale information into industrial-scale inventories. Existing scaling methods, engineering approximations, simulations, and empirical relations, have been applied in isolated studies but lack a unified, generally applicable structure. Laboratory data typically shows inflated electricity demand, excess solvent use, limited separation performance, and batch-related losses, producing non-linear deviations when extrapolated to

industrial conditions.

This study develops a systematic framework for scaling production steps within pLCA. We combine a structured review of upscaling approaches with an assessment of the physical and operational mechanisms driving scale-dependent changes. A critical-point analysis identifies the flows most affected by scale, including energy use, solvent and reagent consumption, waste generation, and infrastructure effects, clarifying which parameters require adjustment.

A general upscaling strategy is introduced based on dominant unit operations such as heating, cooling, drying, and solvent recovery, supported by a calculation tool that derives energy and fuel demand from low-TRL data and enables scenario building. Minimum data requirements (stoichiometry, yields, solvent and reagent flows, thermal loads, waste streams) provide a consistent basis for extrapolation.

Sector-specific mechanisms in petrochemical, biochemical and pharmaceutical systems are incorporated, accounting for thermodynamic integration, mass-transfer limitations, bioreactor behavior, high-purity separation and utility-intensive operations.

The resulting framework offers a transparent and reproducible pathway to construct industrial-scale inventories when only early-stage information is available, improving robustness, comparability and interpretability in pLCA.

5.02.P-Tu414 Toward the ecodesign of emerging technologies: a collaborative bibliographic tool for technology upscaling modelling in LCA

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The upscaling of emerging technologies is a multifaceted socio-technical phenomenon covering technological development and deployment in society to meet societal imperatives (e.g. sustainability). It has been characterized in previous research by five archetypes: upsizing from laboratory to industrial scale (archetype 1), mass producing related to industrial engineering (archetype 2), reaching a level of cumulated service (archetype 3), integrating a complex system (archetype 4) and down-limiting related to planetary boundaries (archetype 5). Each upscaling archetype encompasses a specific industrial system vision, referring to a great variety of lifecycle inventory modelling. To help LCA practitioners and designers collaborate on the upscaling modelling, this research paper presents a methodology supported by a literature management tool: the upscaling archetype (UA) by Engineering Field (EF) matrix. A case study on the photovoltaic sector is presented to show the diversity of available models. The use of the UA-EF by LCA practitioners and technical engineers could induce the design team to take ownership of the upscaling archetypes and help generate shared representations and LCA practices. This tool is intended for distribution to research institutes and companies to be regularly updated and strengthen interdisciplinarity between engineering fields and LCA.

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5.02.P-Tu415 A Joint Conceptual and Computational Structure for Dynamic and Prospective LCA

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As sustainability challenges intensify and decision-makers increasingly seek to anticipate future consequences, a growing number of studies are extending LCA to account for temporal aspects. However, the terminology utilized to describe this kind of studies remains inconsistent. In particular, the distinction between “prospective LCA” and “dynamic LCA” is often unclear, and the underlying computational approaches are not always clearly defined. This lack of clarity limits effective communication, comparison of studies, and the development of standardized and consistent tools.

We set this work specifically to clarify the differences between “prospective” and “dynamic” LCA studies and focus on representing this through a matrix-based computational structure in parallel with the discourse on semantics. First, we mapped the most commonly utilized definitions of prospective and dynamic LCA, particularly focusing on the standard inventory and impact assessment phases. Second, we expand the classic matrix notation to reflect all temporal dimensions that can potentially be captured in LCA. Third, we map the relations between the existing tools that allow prospective and/or dynamic LCA and the classification we

propose.

We summarize a set of definitions and a classification of dynamic and prospective LCA studies that prioritize the computational distinctions. We discuss the potential and limitations in the context of existing approaches and tools, and the associated practices. We highlight that trade-offs can arise between comprehensiveness, computational effort, and data requirement. We discuss the implications of accounting for the temporal aspects in all the elements of the classic matrix-based calculation approach to provide clear distinctions. We believe that this pragmatic approach should be used in parallel with the discourse on semantics. The focus is therefore on classifying a model based on its structure rather than based on its name here. The classification can facilitate modelling choices to match the data availability and the needs of decision-makers.

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5.02.P-Tu416 Building a prospective, scenario-based environmental assessment of future European battery recycling by coupling Material Flow Analysis and Life Cycle Assessment

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Driven by the increase in electric vehicles, portable electronics, light electric mobility, and stationary energy storage, battery waste in Europe is increasing rapidly and becoming more complex due to differences in chemistries, applications, and recovery pathways. Projecting the environmental impacts attributed to this waste stream and identifying potential environmental trade-offs is needed to shape policy, but these impacts are highly sensitive to several key parameters that are not typically included in traditional Life Cycle Assessments. Changes in battery demand, development of new battery chemistries and recycling technologies, alternative second-life pathways such as refurbishment and reuse, as well as decarbonisation of the energy grid, all have major implications for the environmental performance of future recycling systems.

This study aims to cover these dynamic conditions by combining a scenario-based material flow analysis model with prospective Life Cycle Assessment. We cover the main battery types used in Europe across all applications: lead-acid, lithium-ion, nickel-cadmium, nickel-metal hydride, and alkaline batteries. A hierarchical material flow model tracks the end-of-life flows of products, components, materials, and elements across three scenarios - Business As Usual, Increased Recovery, and Circularity. These flows are integrated into a prospective LCA using dynamic background inventories generated with PREMISE, which applies energy- and industry-system transformations from integrated assessment models to reflect future electricity mixes, fuel use, and material production. Integrating both dynamic foreground flows and background conditions in this way allows for consistent comparison between system parameters and makes it possible to isolate the drivers most responsible for future variability in environmental impacts.

Preliminary results show that the greenhouse gas impacts of recycling lithium-ion batteries are projected to exceed the impacts of all other battery types combined by 2040 in all three scenarios. Sensitivity analysis will identify the most influential system parameters, with a particular focus on how shifts in lithium-ion chemistries affect recoverability and environmental impacts.

Disclaimer/Disclosure:

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5.02.P-Tu417 Uncertainty and Sensitivity of Scenario Choices in Prospective LCA

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Uncertainty is recognised as inevitable in prospective Life Cycle Assessments (LCAs), and scenarios are a widely suggested approach to address uncertainties about future developments. Recent methodological advancements have been made to systematise the generation of prospective background systems. However, it is unclear how the use of such future scenarios affects the comparison between technologies. This study investigates the influence of scenario-related uncertainty on the results of prospective LCAs.

Using a novel LCA tool, we assess the same set of technologies under different future scenarios generated in Premise. The modelling spanned across 19 future scenarios for the scenario years 2030, 2040, and 2050. Four technologies from different sectors were chosen as case studies to enable analysis of potential sector-related differences: electricity, transport, steel and agriculture. Each technology was modelled for two countries to analyse potential geographical differences. For continuity and comparability, the technologies were modelled for one European and one Asian country. The scenario results were analysed to identify which conditions lead to ranking one alternative as better than the other, and how consistent the evaluation was across future scenarios.

The results indicate a sector-specific sensitivity for the climate change impact category, depending on which scenarios are included in the analysis. This sensitivity is highlighted by the example of electricity, where the choice of scenario can shift the ranking of the regional impact scores. This effect was not observed for the transport, steel and agricultural sectors. Consequently, the choice of scenarios introduces an additional source of uncertainty, as the ranking of alternatives becomes dependent on the selection of scenarios. In a decision-making context, this means that scenario choices could lead to different conclusions.

This study highlights and analyses the existing gaps in knowledge about the implications of scenario choices for prospective LCIA results. This work further emphasises the importance of using multiple scenarios to explore a range of possible futures, and to be aware that outcomes may vary substantially depending on scenario choices. Decisions supported by prospective LCA should consider the scenario-dependency of results as a critical dimension of uncertainty that can influence the robustness and relevance of conclusions.

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5.02.P-Tu418 Prospective Life Cycle Assessment of Novel Nanofiltration Membrane Manufacturing Methods for Sustainable Scale-Up

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As water scarcity becomes more severe, membranes have emerged as a major technology for water treatment and purification, acting as a selective barrier that allows water to pass while retaining contaminants. Among them, nanofiltration (NF) membranes specifically remove small organic molecules and multivalent ions. However, their manufacturing relies on fossil-based raw materials and solvents. Novel fabrication techniques have shown potential as sustainable alternatives to conventional processes, yet they are still in early development stages. In this work, a prospective Life Cycle Assessment was combined with process modelling to evaluate the environmental performance of novel fabrication methods, ranging from laboratory scale to state-of-the-art techniques, and study their scalability potential. The manufacturing process of a hollow fiber nanofiltration membrane module consists of four steps: fiber support fabrication, nano coating, drying, and module assembly. Since the last two steps are always required for membrane conditioning and module preparation, the comparison was focused on the first two steps. Three technologies were evaluated for the first step and two for the second. The results were quantified per 1000 m², and the methods with lowest environmental burden for each step were identified. Implementing emerging technologies, compared to the conventional process, led to a CO₂ reduction of up to 75% in the fiber support fabrication and up to 46% in the coating step. Considering all four steps of the process, the total CO₂ emissions were reduced by up to 37%. Hotspots of the process were identified, and a sensitivity analysis assessed their influence on the overall environmental impacts. The parameters studied included electricity source, water use, glycerine use, pH conditions and solvent recycling. These insights will help membrane researchers and industry manufacturers identify critical improvement areas and move toward more sustainable membrane production.

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5.02.P-Tu419 Precision in Prediction: Mitigating Material-Related Uncertainty in Prospective Product Carbon Footprinting

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Early-stage prospective life cycle assessment is valuable for supporting product development decisions before design choices become fixed and costly to modify. Because material production often dominantly contributes to cradle-to-gate product carbon footprint (PCF), early uncertainty in provisional material type, supplier choice and recycled content can strongly influence prospective PCF estimates. At the same time, the growing integration of automated assessment tools into digital design workflows requires PCF calculations to rely on minimal early design information. The approach developed here is demonstrated using mechanically engineered products as a case study.

This study addresses the challenge of quantifying and transparently representing early-stage PCF uncertainty when only limited information is available. It investigates how uncertainty evolves as additional inventory data becomes accessible and whether automated introduction of new information layers can reduce it.

A multi-layer automated framework is developed that sequentially incorporates: (1) proxy material information and estimated net mass; (2) manufacturing processes predicted from historical data; (3) statistically derived scrap-rate distributions for the predicted process; and (4) geometry-based scrap estimation. At each layer, uncertainty in material properties, scrap behavior and process characteristics is represented through probability distributions and propagated to the PCF via Monte Carlo simulation, enabling a structured evaluation of how uncertainty evolves as the LCI becomes more detailed.

It is hypothesized that uncertainty reduction in automated early-stage PCF estimation depends not only on adding new information but on how each layer interacts with and propagates assumptions from earlier stages. Geometry-based scrap estimation is expected to refine uncertainty ranges more reliably where applicable, as it introduces part-specific geometric constraints that depend less on upstream predictions.

This research contributes a transparent approach for automated early-stage PCF estimation that incorporates automated uncertainty quantification and evaluates how uncertainty evolves across sequential data layers, supporting prospective LCA in increasingly digital engineering environments where design decisions must be made with incomplete early-stage information.

5.02.P-Tu420 Dynamic-prospective or time-explicit life cycle assessment: unite two novel approaches

Ladislav Lang-Hogrefe, JOANNEUM RESEARCH, Austria

Dynamic and prospective methods are one of the largest growing research areas in life cycle assessment (LCA). Time differentiating approaches promise to fill gaps in the lacking accuracy of static average and data collected in the past. While previous methods had either dealt with the temporal evolution of transition dynamics or with the temporal distribution of process dynamics, recently two developments have arisen that cover both aspects.

Time-explicit LCA with *bw_timex* includes arbitrary temporal distributions of process flows in front of the evolution of prospectively modified background databases. Dynamic-prospective LCA with *Prosperdyn* develops transition paths for industry systems transforming towards climate neutrality. Although both developments consider absolute and relative temporal information as well as dynamic impact assessment, significant differences occur, which concede both their individual field of application.

The current work attempts ascertaining which of the two software packages suites better for different applications. Areas of interest are biomass growth, energy and industry transformation activities or delayed emissions of carbon capture. The flexibility in relative time information is a great advantage of *bw_timex*. *Prosperdyn* considers the timing of infrastructure deployment separately and avoids depreciation of construction emissions.

In conclusion, the parallel development bears witness of the relevance of a combined dynamic prospective method. Two methods try to fill quite similar research gaps. At this point, there are two possible solutions for continuing methodological research: either allocating distinct use cases to the respective software or finding a sufficient way to integrate the advantages of one method into the other for providing one joint method.

5.03 Chemicals, Dyes and Textiles: Advancing Toxicological Assessments and Life Cycle Analysis for a Safe and Sustainable Future

5.03.T-01 Benchmarking Environmental Impacts in the Safe and Sustainable Design of Chemicals

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The introduction of the Safe and Sustainable by Design (SSbD) framework has emphasized the importance of conducting early-stage environmental sustainability assessment – through Life Cycle Assessment (LCA) – to anticipate environmental burdens of chemicals and materials before they enter the market. This has led to efforts to overcome the limited availability of data for constructing robust early-stage Life Cycle Inventories (LCIs), for example by estimating energy and mass balances, or developing streamlined tools for preliminary assessments of emerging technologies.

Nevertheless, meaningful early-stage assessments depend on the definition of an appropriate benchmark, which is a reference level of environmental performance against which the impacts of a novel chemical or technology are compared. While defining a benchmark is relatively straightforward for LCAs of existing products, the definition of the benchmark for chemicals that assist early-stage assessments is much more challenging.

The goal of this study is to explore how impact values vary across chemicals to establish a benchmark that can serve as a basis for applying the SSbD framework to early-stage design of chemicals. First, we selected 71 organic base chemicals from the related BAT Reference Documents for Large Volume Organic Chemicals (BREF). We calculated the impacts of such selected chemicals, in terms of 1 kg of chemical considering a cradle-to-gate system boundary using EF3.1 method. Third, for each impact category, we compiled the impact values of all the 71 organic base chemicals, identified the full range, ranked the results and split them into quartiles.

This benchmark can ultimately help to – among other - (1) evaluate for which impact categories the cumulative distribution is flat (no impact differences) or vary diverse (steep), (2) understand the (molecular, process, factory) "features" driving the differences, and (3) optimise the innovation towards areas that matter most, benchmarked against the context of the worst-performing alternatives.

This study highlights the need for further discussion on the information needed to establish robust benchmark, including choices of system boundaries and functional unit considerations. Future works will be dedicated in testing and adapting such classes of performances and proxy references, and explore the possibility to create classes of performances for the safety component of the SSbD framework.

5.03.T-02 Potential of Cyanobacterial Pigments for Sustainable Applications in Textile Dyeing

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Growing awareness of the environmental and health hazards posed by synthetic colourants has renewed interest in natural substitutes across various industries, such as cosmetics, food, and textiles. Cyanobacteria are a group of photosynthetic microorganisms known to produce colourful natural pigments as chlorophylls, carotenoids and phycobiliproteins, which have demonstrated potential for large-scale industrial applications [1,2,3], leading to a more sustainable future. This study evaluates the feasibility of using pigments from cyanobacteria as sustainable colourants for the textile industry.

Cyanobacteria strains were obtained from LEGE-Culture Collection and cultivated in Z8/ Z8-TM 25 medium, supplemented with Vitamin B12. Textile dyeing experiments were conducted on both synthetic and natural fibres, considering the pigment content in the aqueous extracts of each strain. Dyeing was performed with a 1:10 bath ratio, using NaCl as an exhausting agent, under a 120-minute cycle at 40°C. A post-dyeing fixation process was applied to enhance colour retention. The dyed fabrics underwent quality control assessments for colour reproduction and fastness to rubbing, washing, water, and perspiration, following European Standards (UNE-EN ISO) for textiles.

In general, the results suggest a better dyeing performance of the studied pigments in the semi-synthetic fibre than in the natural fibre, indicating a higher affinity for the semi-synthetic fibre. According to the classification assigned to these pigments, the results are in line with what is expected for direct dyes. More promising colours were obtained with marine strains, for both viscose and cotton. Regarding colour change assessment, industrial

laundering had the worst results in colour fastness, for both marine and freshwater strains. In general, good results were obtained in the staining assessment for the fibres tested, with values comprised between 3 (fair) and 5 (excellent).

In summary, cyanobacterial pigments show considerable promise as sustainable substitutes for synthetic dyes in the textile industry, provided certain conditions are met. The method currently employed already constitutes a more environmentally friendly approach to textile dyeing. However, additional optimisation, especially through the incorporation of alternative dyeing auxiliaries, may further improve the efficiency and overall performance of this technique.

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5.03.T-03 Toxicity of the Anthraquinone Dye Alizarin to Early Life Stage Zebrafish

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Natural and anthropogenic chemicals are an essential part of modern society, but there are growing concerns about environmental contamination and its effects on organisms. As a result, production and use of more sustainable materials has become a focal point in many industries. This is especially true in the textile industry where there is increasing focus on use of natural dyes as ‘safer’ alternatives to synthetic dyes. One of the oldest natural dyes, alizarin, has emerged as a potential candidate for use in waterless dyeing processes. A small number of studies have shown that alizarin might not be a safe alternative to synthetic dyes, but additional research is needed to assess its toxicity. In this study, zebrafish (*Danio rerio*) embryos were exposed to alizarin by microinjection, and developmental toxicity and associated molecular mechanisms were investigated. Exposure to alizarin resulted in non-monotonic increases in mortality. Additionally, the proportion of embryos exhibiting one or more developmental malformations (i.e. spinal curvature, yolk sac and pericardial edema) followed the same non-monotonic trend. Dose-dependent reductions in body length, eye area, head area, yolk area, and cranial melanophore count were also observed. In a behavioural assay, exposure to alizarin caused dose-dependent increases in average swimming velocity that persisted through post-stimulus recovery periods. Mechanistically, mRNA abundance of *cyp1a* was increased in embryos exposed to alizarin. Alizarin also activated the zebrafish AhR2, in vitro. Exposure to alizarin also increased mRNA abundance of the oxidative stress response genes *gpx1a*, *sod1*, *gst*, and *cat*. These results suggest that embryotoxicity of alizarin is at least in part mediated by the AhR, with contribution from oxidative stress. Additional work is ongoing to assess the toxicity of other anthraquinone dyes to early life-stage zebrafish.

5.03.T-04 Safe-by-design: Searching for Non-toxic and Non-mutagenic emodin derived dyes

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Dyes play an important role across various industrial sectors. Although many synthetic dyes are available, there is increasing interest in replacing them with natural alternatives. Emodin, a natural dye extracted from the fungus *Cortinarius sanguineus*, shows high technological potential for waterless dyeing methods such as supercritical CO₂ (sc-CO₂). However, emodin is highly toxic to aquatic organisms and mutagenic due to DNA intercalation. Chemical modification of emodin (derivatization) is a promising strategy to reduce or eliminate these negative effects. This study evaluated the aquatic toxicity and mutagenicity of eight emodin-derived dyes to identify promising natural-based alternatives. Emodin derivatives (purity >98%) were provided by the Colour4CRAFTS project. Acute toxicity was assessed through concentration–response tests with *Daphnia similis* (48 h, OECD 202) and zebrafish embryos *Danio rerio* (96 h, FET, OECD 236). Tests included solvent and negative controls and were approved by the UNICAMP Ethics Committee. Mutagenicity was evaluated at each compound's maximum solubility using the Microplate Agar (MPA) version of the Salmonella/microsome assay with TA1537, TA100, YG1041 and TA98 strains, with and without metabolic activation (S9). TA1537 strain was included due to its known sensitivity to anthraquinones. All derivatives were less toxic to *Daphnia* than emodin. For zebrafish embryos, five derivatives tested also showed reduced toxicity. Emodin was mutagenic only in TA1537 (+S9) and negative in the other strains. Among the derivatives, EMD1, EMD4 and EMD8 exhibited a mutagenic response similar to emodin in TA1537 (+S9). EMD4 additionally showed

mutagenicity in TA100 ($\pm$ S9) and TA98 (?S9), while EMD8 was mutagenic in TA100 and TA98 (?S9), indicating the derivatization led to other forms of interaction with DNA. EMD2, EMD3, EMD5, EMD6 and EMD7 were non-mutagenic in all strains. Derivatization successfully reduced acute toxicity for all compounds except EMD8, and five derivatives lost the ability to induce mutations. EMD3 and EMD7 were the most promising dyes, showing no acute toxicity to *Daphnia* and no mutagenicity. Ongoing fish embryo tests are being done and then we will be able to verify if it is possible to obtain an emodin derivative that is not toxic to both, organisms and not mutagenic. This work illustrates the safe-by-design concept and reinforces the importance of toxicological evaluation in the development of new dye products.

5.03.T-05 In Silico Approaches for the Prediction of Climate Indicators for Life Cycle Impact Assessment *Arianna Sgariboldi, Nicola Chirico and Ester Papa, DiSTA, University of Insubria, Italy*

Life cycle assessment (LCA) is a valuable tool for analysing and managing the environmental impact of substances throughout their life cycle. One of the four stages that define LCA is life cycle impact assessment (LCIA), during which various climate change-related indicators are analysed and quantified, such as global warming potential (GWP) and ozone depletion potential (ODP). The Safe and Sustainable by Design (SSbD) framework suggests the use of in silico models based on machine learning techniques, such as quantitative structure–activity relationships (QSAR), to address potential data gaps relating to the activities and/or properties of existing and new chemicals and chemical alternatives. In this study, QSAR models were developed to estimate the most used indicators and properties in climate assessments, thereby helping to overcome the lack of experimental data for existing and new chemicals. QSAR models were developed using multiple linear regression by means of ordinary least squares (MLR-OLS) and variable subset selection (VSS) to predict GWP, ODP and removal efficiency for a large dataset of around 700 chemicals, divided into 20 different structural classes (mostly halogenated), compiled from the latest Scientific Assessment of Ozone Depletion report published by the World Meteorological Organization (WMO) in 2022. The best QSAR models were selected based on multiple metrics of goodness of fit, robustness, and predictive ability, as well as an evaluation of the applicability domain. These models have R^2 values ranging from 0.75 to 0.84 and Q^2_{ext} values ranging from 0.70 to 0.88 and have proven to be reliable tools for predicting climate indicators. The molecular descriptors selected in the best models mainly encoded electronic and geometric properties, as well as the presence of specific atoms and fingerprints. These new QSAR models are available in the free software QSAR-ME Profiler 2025 and can be easily applied to predict the properties of new chemicals, thereby reducing data gaps in environmental assessments. Furthermore, they can be incorporated into sustainability evaluation processes to support the selection of environmentally preferable alternatives and to provide valuable input for the LCIA stage of the LCA, as well as for the SSbD framework.

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5.03.P Chemicals, Dyes and Textiles: Advancing Toxicological Assessments and Life Cycle Analysis for a Safe and Sustainable Future

5.03.P-Mo334 C.I. Direct Blue 218 induces micronuclei in somatic cells and DNA damage in sperm of a model invertebrate in in vivo experiments

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C.I. Direct Blue 218 (DB218) is a copper-chelated dimethoxybenzidine-based azo dye used in textile and paper industries. It was classified by IARC as class 2B as there are few and conflicting data available. Also no data on its aquatic toxicity was found in the literature. The invertebrate *Parhyale hawaiiensis* has been recognized as a new model in ecotoxicology, is easily cultured in the lab and can provide insights on different endpoints including mortality, reproduction inhibition and genotoxicity in somatic and germ cells. Its ability to digest cotton fibers can also be used as an alternative route of exposure. This study evaluated the effects of DB218 (purity >95%) in *P. hawaiiensis* observing mortality (via water) and reproduction (via dyed cotton ingestion). Its genotoxicity was evaluated by the micronuclei test in somatic cells (hemocytes) and in sperm cells (DNA damage). To complement the bacterial mutagenicity data a test with an alternative strain of *Salmonella* (YG1041) that is very sensitive to azo dyes was conducted. Acute toxicity (96 h) with neonates of *P. hawaiiensis* followed a standardized protocol. A chronic test was conducted with 60 organisms aged 80 days to assess the reproductive

effects of the ingestion of dyed cotton fibers. After 40 days the number of neonates per female was recorded and compared to the historical control. For bacterial mutagenicity, DB218 was tested in the miniaturized version of the Salmonella/microsome assay (Microplate Agar, MPA) with the YG1041, without and with S9 (5, 10, and 30%). Micronuclei frequency was assessed in hemocytes of 30 adults of *P. hawaiiensis* after 96 h exposure. Slides were stained with 10% Giemsa, and 500 cells per organism were counted. For DNA damage, the comet assay in sperm cells was performed after 96 h exposure of 16 adult males. One hundred comets per gel were analyzed (%DNA in tail) using Comet Score 2.0. No acute toxicity was observed in *P. hawaiiensis* and ingestion of DB218 dyed cotton did not affect reproduction. DB218 showed no mutagenicity in YG1041 without and with metabolic activation (S9). However, DB218 significantly increased micronuclei frequency in hemocytes and caused sperm DNA damage. This work provides evidence that DB218 is a possible germ cell mutagen. Experiments are being conducted using enzyme biomarkers to help elucidate its mode of action. *P. hawaiiensis* model seems to be a promising tool in eco/genotoxicology.

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5.03.P-Mo335 Integrating Cyanobacterial Pigments into Cellulosic Textiles To Enhance Circularity and Sustainable End-of-Life

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The end-of-life management of textiles is a critical environmental challenge, particularly for cellulosic fibers, which can be designed to enhance biodegradability. Using biobased colorants and coatings from cyanobacteria offers dual benefits: sustainable pigmentation and facilitation of fiber breakdown in composting or soil. These bio-based approaches also reduce the ecotoxicity risks associated with conventional synthetic dyes, which are often persistent, bioaccumulative, and harmful to soil and aquatic organisms. Furthermore, fiber configurations, including yarn structure and fabric density, can influence microbial accessibility and enzymatic depolymerization, linking material design directly to environmental safety and sustainable end-of-life outcomes. This review synthesizes recent advances in applying cyanobacterial pigments and biomass in textile production, highlighting their use as natural colorants and functional coatings with low ecotoxicological impact. It examines how textile configurations influence biodegradation and environmental safety, considering the interplay between fiber type, structure, microbial susceptibility, and potential contaminant release. Particular attention is given to phycobiliproteins and whole biomass applications, focusing on their binding efficiency to cellulosic substrates and compatibility with biological degradation.

Cyanobacterial pigments have been reported to provide effective and relatively stable coloration of cellulosic fibers, while potentially minimizing ecotoxicological concerns. Coatings incorporating pigment extracts or whole biomass appear promising in enhancing microbial degradation, although further empirical validation is needed. Overall, the reviewed literature indicates that the combination of natural bio-based pigmentation with intentionally designed fiber configurations may facilitate accelerated biodegradation and reduce environmental risks, without substantially compromising material performance during use, though these effects remain context-dependent and warrant additional investigation.

Integrating cyanobacterial pigments and biomass with fiber configurations offers a promising pathway toward sustainable, circular textile lifecycles. By linking pigmentation, fiber design, and ecotoxicological considerations, these bioinspired approaches support environmentally responsible end-of-life scenarios and a transition toward a safer, more circular textile industry.

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5.03.P-Mo336 Microbial Decolourisation of Commercial Textile Dyes

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Synthetic dyes used in textile processing are increasingly recognised as environmental contaminants of concern due to their aromatic complexity, persistence and potential ecotoxicity. Current EU regulatory developments are placing growing pressure on the textile industry to mitigate dye pollution. In this context, biological decolourisation strategies offer promising low-impact alternatives for treating dye-containing effluents. This study assessed the ability of bacterial and fungal isolates obtained from composting environments to decolourise four commercial textile dyes. Bacterial and fungal isolates previously recovered through a cellulolytic culturomics workflow were tested against four industrial dyes. Assays were performed at two dye concentrations (0.03 and 0.06 g/L) in Nutrient Broth, using incubations at 28 °C (bacteria) and 25 °C (fungi). Decolourisation was quantified spectrophotometrically at dye-specific wavelengths over seven days. For fungal assays, both live and autoclaved fungal biomass were evaluated to distinguish the contributions of active biological processes and non-active mechanisms to colour removal. All assays included triplicates and uninoculated controls. Bacterial isolates exhibited significant variation in decolourisation efficiency across dye types ($p < 0.05$). Several strains exceeded 80% decolourisation for Red, Yellow, and Navy dyes. Fungal isolate *Mucor circinelloides* F-CL4 demonstrated similar efficiencies between live and autoclaved biomass for certain combinations. Decolourisation of the Black dye was limited under all tested conditions. These findings indicate that multiple mechanisms contribute to colour removal. Environmental microbial isolates, including fungal strains such as *Mucor circinelloides* and bacterial strains from genera such as *Bacillus*, *Glutamicibacter*, and *Glycomyces*, demonstrated measurable decolourisation capacity across a range of commercially relevant textile dyes. Although removal efficiencies varied markedly by dye structure, several isolates achieved high levels of colour reduction. This indicates that dye removal proceeds through multiple mechanisms, with biosorption likely dominating for several dye classes. Collectively, these findings highlight the potential of environmental microorganisms as low-impact biological tools for the treatment of dye-containing textile effluents and support the advancement of more sustainable, biologically based strategies for mitigating dye pollution.

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5.03.P-Mo337 PFAS as the “knot in the yarn” of textile circularity

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Per- and polyfluoroalkyl substances (PFAS) are a category of chemical commodities with orthogonal application across many industrial and economic sectors. The textile industry is not an exception to this, as PFAS have a ubiquitous presence across the textile value-chain and the life-cycle of textile products. Despite recent pushes towards PFAS-free textiles (either voluntary or regulatory-driven), PFAS impregnation (e.g., polytetrafluoroethylene, side-chain fluorinated polymers) remains the main strategy for the manufacturing of functional and occupational textiles with water-repelling or fire-retardant properties. However, even if the removal of PFAS as textile components is achieved, per- and polyfluorinated chemicals are still expected to be a common occurrence across the life-cycle of textiles, either as contaminants of raw materials (e.g., per and polyfluorinated pesticides in natural fibers), chemical finishing or coating of garments or via cross-contamination from different sources during manufacturing and transport.

Textile waste is a massive global challenge on its own, but current waste management practices of PFAS-containing textile waste (i.e., landfilling and incineration) have the potential to aggravate this environmental problem by serving as sources of PFAS dissemination into the ecosystems via soil percolation, leaching and volatilization. European sustainability policies and the textile waste management sector have been pushing for the reuse, recycling and valorization of textile waste through greener and bio-based processes (e.g., biological/chemical recycling, biorefineries, biodegradation), but the biological recalcitrance of PFAS turns them non-compatible with the typical circularity of these processes.

In this platform presentation, we will showcase our ongoing research on the microbial defluorination (i.e., the cleavage of the highly stable carbon-fluorine bond) of persistent fluorinated pollutants, including PFAS, and reflect how defluorinating microbes and their enzymes can be critical assets in the design of avant-garde bioprocesses for textile waste mitigation, recycling and valorization.

Disclaimer/Disclosure:

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5.03.P-Mo338 Hybrid Flow Chemistry Designs Reduce the Environmental Impacts of Pharmaceutical Production

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The pharmaceutical sector urgently needs to decarbonize. In 2015, its emission intensity was ~55% higher than the automotive industry's. One proposed strategy is the use of flow chemistry. Typically, pharmaceuticals are produced using batch chemistry, in which production occurs over discrete batches and steps. This is a slow, costly, and waste-generating process that drives up emissions. In flow chemistry, production occurs in one continuous stream. Where feasible, flow chemistry has several advantages over batch, including an improved safety profile, faster throughput, smaller physical footprint, reduced waste, and a lower demand for solvents and reactants. While the advantages of flow chemistry are evident for single-step reactions, the implications of transitioning from batch to flow are less clear for multi-step syntheses. Using life cycle assessment, we evaluate the environmental impacts of producing an active pharmaceutical ingredient (API) under batch, flow, and hybrid configurations to determine whether flow consistently reduces environmental impacts in pharmaceutical production. To this end, we assessed trazodone hydrochloride, an antidepressant medication, which is produced over three phases. Using primary data from the manufacturer Angelini Pharma, we assessed four configurations: Batch, Flow, Batch–Batch Optimized, and Flow–Batch Optimized. These vary in Phase 1 (batch or flow) and Phase 2 (batch, flow, or optimized batch with improved solvent use/recovery), while Phase 3 (finishing) remained the same. Flow, Batch–Batch Optimized, and Flow–Batch Optimized all show considerable emissions reductions across every impact category, except freshwater ecotoxicity. The hybrid alternative Flow–Batch Optimized has the best environmental performance overall, with reductions around 18% for the climate change impact category compared to the baseline. This shows that, contrary to expectations, a fully continuous system is not always optimal; a hybrid system of flow and optimized batch might yield better environmental outcomes. Across all alternatives, the main contributing factors to climate impacts include supply-chain factors such as the production of precursor chemicals, followed by hazardous waste incineration, solvent recovery, and solvent use. Our results show no universal advantage for flow over batch; only thorough assessment of different configurations can reveal the best option. A hybrid configuration might be the way forward for API production.

Disclaimer/Disclosure:

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5.03.P-Mo339 Fast-screening tool for early-stage environmental assessment of pharmaceutical synthesis route design

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With more innovative active pharmaceutical ingredients (APIs) developed and applied, synthesis processes become more complicated, increasing the environmental burdens of the pharmaceutical production. As the key element of the drug design, synthesis route design is very crucial for integrating the environmental sustainability at the earliest stage. Early-stage API development often involves low technology readiness, with limited synthesis data and numerous assumptions in scaling, resulting in high uncertainty in environmental impact assessments. While some screening tools exist to conduct a simplified assessment, the specific capability of these tools on route screening is not clear, and pharmaceutical companies each choose a tool including different features. The aim of this research is to decide an optimal tool that consolidates key methodological elements from both literature and practice to facilitate the early-stage sustainability assessment of API synthesis routes in both academic and industrial contexts.

This study first identified the existing assessment tools by conducting a literature review. Considering

prospective assessment is also an important aspect of synthesis route screening, we conducted two rounds of literature searching on screening tools and prospective assessment tools respectively. Second, we distributed online surveys to pharmaceutical companies to collect information on screening tools used, environmental considerations, and expectations for an optimal tool. Third, a set of criteria was determined from both literature and surveys to assess and compare the functionality and characteristics of each identified assessment tool. Results show that the performance of assessment tools under different criteria differs a lot. In terms of impact coverage and life cycle phase coverage, tools such as LiSET and FineChem extend their system boundaries, allowing for a more comprehensive evaluation. Meanwhile, CHEM21 and GCIPR focus only on the reaction stage, providing process-level insights without full life cycle interpretation. FLASC and iGAL provide route-level hotspots while able to compare different processes or routes. In this case, a combination of tools is more suitable than a single tool for synthesis route design. The study will finally decide which tools to combine together to frame a suitable screening tool. During the presentation, a case study will be presented to show the applicability of this final early-stage screening tool.

5.03.P-Mo340 A Critical Review on Environmental Sustainability Assessment of Decontamination Processes in the Supply of Medical Devices and Pharmaceuticals

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Decontamination processes are vital across sectors such as food, biotechnology and in particular the supply of medical devices (MDs) and pharmaceuticals to ensure sterility and safety. Their application minimizes the risk of contamination, thereby safeguarding product quality and public health.

However, these processes are often energy- and resource-intensive, generating diverse waste streams, resulting in notable environmental impacts. Life Cycle Assessment (LCA) provides a standardized framework to quantify such impacts. Existing LCA studies on decontamination show that the contribution of decontamination to the overall environmental impact often varies due to e.g., the choice of decontamination process and differences in LCA methodology. It is therefore vital to provide a thorough overview of the current state of LCA studies on decontamination and to identify key differences among them.

This study presents a comprehensive review of the extant literature on environmental sustainability assessments of decontamination processes, focusing on the supply of MDs and pharmaceuticals. The food sector is also included due to strong technological parallels. The primary objective of this study is to provide insights into the available LCA studies to highlight methodological differences within the four-step framework defined in ISO14040. To this end, 2349 studies were screened, of which 70 fulfilled the inclusion criteria for detailed analysis. These were categorized by the type of decontaminated product: products, production equipment, production environment and waste. For each category, technology maps were developed to classify the various decontamination techniques (e.g., chemical, thermal), providing an overview of documented techniques. Studies will be compared regarding goal and scope, data inventory and impact assessment methodology. The impact assessment analysis will ascertain the contribution of the decontamination to the total life cycle impacts per considered product category. Furthermore, innovative technologies will be benchmarked against conventional ones. One preliminary result shows that foam flow cleaning can reduce the carbon footprint by up to 95% compared to traditional Cleaning-In-Place. The outcomes of this review will facilitate the provision of guidance for future LCA studies addressing decontamination processes and establish a fundamental basis for subsequent research endeavors regarding the environmental sustainability of these processes.

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5.03.P-Mo341 Developing a Freshwater Ecotoxicity Characterization Factors Database for Cosmetic Ingredients: MSS-HC5 and Clustering Approaches

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The EcoBeautyScore (EBS) Association has developed a harmonized environmental footprinting and scoring system for cosmetic products, aligned with the EU Product Environmental Footprint (PEF). Since 2025, this

scoring system has been deployed across Europe to enhance transparency for consumers.

One of the main methodological challenges concerns freshwater ecotoxicity, especially during the end-of-life stage of cosmetic formulas. The EF 3.1 database covers only a limited share of cosmetic ingredients and often lacks robust characterization factors.

To address these data gaps, EBS developed two methodological innovations.

The first is the MSS-HC5 method for deriving effect factors (EF). Unlike the PEF approach, which relies on statistical Species Sensitivity Distributions (SSD-HC20), MSS-HC5 uses a streamlined but highly reliable dataset of chronic ecotoxicity values (EC10 or NOEC) across the three standard trophic levels. The lowest chronic value—the Most Sensitive Species (MSS)—is selected and adjusted using a Coverage Factor that accounts for data completeness. When chronic data are missing, acute EC50 values are converted using acute-to-chronic ratios. This approach reduces variability, maintains conservative protection goals, enables a efficient scaling and long-term database maintenance.

The second innovation concerns the estimation of fate and exposure factors ($FF \times XF$). Since full calculation requires many physicochemical parameters, EBS developed a clustering-based read-across method. From a dataset of over 1,100 substances, biodegradability and bioaccumulation were identified as the dominant drivers of variation in log (CF). Four clusters were defined using thresholds for biodegradability and bioaccumulation. Each cluster was assigned a semi-specific $FF \times XF$ value.

The integration of MSS-HC5 with the clustering approach increased coverage of freshwater ecotoxicity characterization factors from about 30% to roughly 60% for the EBS priority ingredients. The updated database also improves applicability for REACH-exempt substances, such as polymers or low-volume ingredients.

In conclusion, the combined MSS-HC5 and $FF \times XF$ clustering methods provide a pragmatic, scalable, and scientifically robust framework for generating freshwater ecotoxicity characterization factors tailored to cosmetic ingredients. This approach strengthens reliability, addresses data scarcity, and supports the operational rollout of the PEF-aligned EcoBeautyScore systems.

5.03.P-Mo342 Differences in the Sensitivity of Freshwater and Marine Organisms in the Acute Toxicity Evaluation of Deep Eutectic Solvents

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Environmentally friendly solvents such as Deep Eutectic Solvents (DES) have attracted growing interest. DES are formed by combining safe, inexpensive components that, through hydrogen bonding, create mixtures with melting points lower than those of the individual substances. Although considered green alternatives, their wide range of formulations requires evaluation of their potential environmental impacts. This study aimed to evaluate the acute toxicity of five DES produced using 1:1 L-menthol (LM) with acetic, propionic, hexanoic, octanoic, and decanoic acids, using the freshwater microcrustacean *Daphnia similis* and the marine amphipod *Parhyale hawaiiensis*. DES were dissolved in culture water under agitation, and five concentrations were tested for each formulation. *Daphnia* tests were conducted using neonates (?24h), with 4 replicates per concentration (10mL of solution and 5 organisms per tube) at 20±2 °C under a 16:8h light: dark photoperiod for 48h. *Parhyale* tests were conducted using neonates (?7d) in 96-well microplates, with 24 replicates per concentration (200µL of solution and 1 organism per well) at 26±2 °C under a 12:12h light: dark photoperiod for 96h. Tests were valid when control immobility/mortality was ?10%. The 50% effective/lethal concentrations (EC50/LC50) were estimated using the drc package in R. For *D. similis*, the DES ranked from most to least toxic based on their EC50 values as follows: LM: Octanoic Acid (0.0002%) > LM: Decanoic Acid (0.0007%) > LM: Hexanoic Acid (0.002%) > LM: Propionic Acid (0.003%) > LM: Acetic Acid (0.005%). For *P. hawaiiensis*, the LC50 values ranked: LM: Hexanoic Acid (0.00004%) > LM: Propionic Acid (0.0003%) > LM: Acetic Acid (0.002%) > LM: Octanoic Acid (0.003%) > LM: Decanoic Acid (0.02%). For the acetic acid formulation, *D. similis* and *P. hawaiiensis* responded in the same range of sensitivity. For the propionic and hexanoic acid formulations, *P. hawaiiensis* was more sensitive, and the opposite was observed for the octanoic and decanoic acid formulations. These findings indicate that DES can be highly toxic (up to LC50 0.00004%) and should be evaluated using both marine and freshwater organisms to avoid underestimating their hazard. Additional tests with fish and algae are underway to complement the toxicity assessment. Overall, although these formulations are considered green alternatives, their toxicity should be determined and incorporated into life cycle assessment of the processes in which they are applied.

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5.04 Complementing and Combining LCA with Other Methodologies for Better Decision Making

5.04.T-01 Including Ecosystems in LCA: the BES Footprint

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Answering the need for harmonized assessment of nature from a life cycle perspective, we present a Biodiversity and Ecosystem Services (BES) footprint to assess products and organisation within a consistent and integrative framework. Building on Life Cycle Assessment (LCA) and the System of Environmental Economic Accounting – Ecosystem Accounting (SEEA EA), it brings together established methods from environmental assessment and Natural Capital Accounting (NCA) to create a unified basis for nature-related evaluation and disclosure. The BES Footprint is structured around two core components: the Biodiversity Footprint, which captures biodiversity risk, and the Ecosystem Service Footprint, which quantifies changes in key ecosystem services. The framework outlines methods for embedding ecosystem-service quantification and biodiversity impact modelling within existing LCA structures such as EF 3.1, LANCA, and BioMAPS, while maintaining alignment with SEEA EA and natural-capital principles. It supports assessments under evolving disclosure and regulatory expectations and provides a basis for consistent nature-related risk and opportunity assessment, stronger governance, and improved investor confidence. It covers essential regulating and provisioning services with the option to include additional services or incorporate improved metrics as they become available. The BES Footprint supports multiple levels of assessment depending on user needs: Level 1 (screening; country-averages and no Foreground data), Level 2 (partial assessment; regionalized data with foreground inputs), and Level 3 (full BES Footprint; combination of global and regionalized data). This tiered structure enables its application across diverse decision contexts, from strategic planning and risk screening to detailed site-specific evaluations. To operationalise these applications, further guidance is foreseen. A systematic comparison of LCA and NCA underpins the BES Footprint, highlighting their complementary strengths and the need for integrated approaches. It addresses key challenges—including spatial and temporal resolution, assumptions and simplifications, and value-chain coverage—and proposes modelling principles that support consistent, scalable assessment of biodiversity and ecosystem services. The application of the BES Footprint follows the LCA phases—goal and scope definition, inventory, impact assessment, and interpretation. These developments form part of the broader A-Track project

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5.04.T-02 Integrating Criticality Indicators into the Life Cycle Assessment Framework: Hydrogen Production as a Case Study

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Environmental Life Cycle Assessment (LCA) traditionally characterizes resource-related damage primarily through the concept of resource depletion. However, this concept does not fully capture broader concerns such as geopolitical, economic, and social constraints that affect resource availability. To address this limitation, it is essential to integrate criticality assessment of raw materials into the LCA framework. This integration allows for evaluating not only physical scarcity but also risks related to accessibility, geopolitical concentration, and market volatility.

In the context of the energy transition, hydrogen is emerging as a key energy carrier, particularly through water electrolysis. Yet, several electrolysis technologies rely on critical raw materials (CRMs), such as platinum group metals in proton exchange membrane (PEM) electrolyzers and rare earth elements in solid oxide electrolysis cells (SOECs). Although these technologies offer strong environmental performance, they also raise concerns about supply risks and long-term resource security. Current environmental LCA methodologies have limited capability to capture such trade-offs.

This study applies an integrated LCA framework that incorporates raw material criticality indicators, especially supply risk metrics, into conventional environmental LCA. The approach links geopolitical supply risk as a midpoint characterization factor with material mass flows within the LCA, aiming to provide results that reflect both environmental impacts and potential supply chain vulnerabilities.

A case study on hydrogen production via these electrolysis technologies is used to illustrate the method. The analysis highlights trade-offs between environmental performance and raw material criticality, offering insights into how decisions favoring environmental benefits may introduce new material dependencies. Integrating criticality indicators into the LCA framework supports more holistic decision-making in energy technology development and resource planning, offering a dual-perspective contribution to sustainable development.

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5.04.T-03 Behaviour Operationalization in Life Cycle Assessments for Decision Making – an Interdisciplinary Framework

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Life cycle assessments (LCAs) are investigating environmental effects of products, technologies and services over their whole lifecycle and are an essential tool when it comes to decision-making in various contexts. Even though many studies – especially of technological devices – attribute large shares of the environmental impacts to the use phase of products, effects and impacts of user behaviour are often neglected or only considered in simplified manners. As there is a lack of standardization in norms and guidelines on the operationalization of behavioural effects, our study develops a framework to integrate user behaviour into LCAs, supporting informed decision-making.

Based on expert interviews with social scientists and LCA practitioners followed by a consecutive deductive content analysis, we identify challenges and opportunities for operationalizing user behaviour in LCAs for the framework development. The proposed framework combines qualitative methods (e.g., interviews, ethnographic methods) with quantitative social scientific data to contextualize LCA results. Clustering approaches (e.g., k-means or agglomerative clustering) are applied to represent different technology adoption and usage behaviours. The integration of user behaviour into LCAs can provide a more comprehensive understanding of the environmental consequences of technology design, enabling decision-makers to make more informed choices about investments, policies, and technological setups. Our study addresses the current lack of standardization and guidance on operationalizing user behaviour in LCAs. By developing a framework that is applicable to various technologies, we aim to support the development of more sustainable products, services, and technological infrastructures. The proposed framework can be used to evaluate the environmental impacts of different technologies with a focus on energy using devices that strongly rely on human-technology interactions (like smart technologies, digital applications etc.)

The framework enhances the accuracy and reliability of LCA results by combining the LCA methodology with social scientific approaches, ultimately supporting decision-making practices. Furthermore, our study highlights the importance of considering user behaviour in LCA studies, as it can significantly influence the environmental performance of products and services, being therefore relevant in strategic decision-making for sustainability.

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5.04.T-04 Integrating planetary boundaries into energy system optimisation models for absolute environmental sustainability assessment: a methodological framework

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Transforming energy systems to address climate change must avoid shifting burdens to other planetary boundaries. Yet most energy models still focus primarily on greenhouse gas reduction, often neglecting broader life-cycle impacts on critical Earth-system processes. This narrow scope can unintentionally shift environmental pressures from climate to land, materials, or ecosystems, even in scenarios deemed sustainable.

To address this gap, we present a modeling framework that integrates prospective life cycle assessment and planetary boundary metrics into a national energy optimization model. The framework first incorporates life-cycle and planetary-boundary metrics into a multi-objective optimization model, then uses goal programming to characterize the different trade-offs in environmental impacts, and finally applies interpretable machine learning to streamline the overwhelming set of trade-offs into system archetypes.

Applied to a 2045 low-carbon scenario for Belgium as a case study, the approach reveals that all near-optimal pathways meet the climate target but exceed planetary boundaries for land use and ecotoxicity under the most permissive downscaling principle, indicating that decarbonization alone is insufficient for absolute sustainability. From the near-optimum space exploration and interpretable machine learning, four distinct system configurations emerge—dominated respectively by electrification, hydrogen, renewable power capacity, or mixed strategies—each achieving similar costs yet distinct environmental trade-offs.

Future extensions of this work should incorporate dynamic demand changes and behavioral adaptations to further align energy systems with absolute sustainability targets.

5.04.T-05 Integrating Life Cycle Assessment with Complementary Methods for Low-carbon Hydrogen

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To reduce greenhouse gas (GHG) emissions according to Paris Agreement, Europe has to become climate neutral up to 2050 and one of the key solutions seems to be energy transition towards low-carbon hydrogen. However, the energy transition presents significant challenges, as it entails diverse impacts, such as social or environmental impacts, that vary across projects and require assessment through suitable methodologies. A commonly used methodology is Life Cycle Assessment (LCA), however not all sustainability aspects are covered through it. This study focuses on the complementary methods used along LCA to identify how the dimensions of sustainability are assessed so far in the low-carbon hydrogen projects in European Union (EU). A systematic review was conducted across Web of Science for studies published between 2015 and 2024. The review comprised of several phases, each of which addressed various limitations. Moreover, whilst the study was initially conceived with a global scope, the area of interest was subsequently limited to the EU to ensure the accuracy of our derivative insights. A comprehensive analysis of the articles revealed significant findings into the coupled methodologies employed in LCA, and the conditions under which they are applied.

Findings of the study prompted a review of 90 methodologies with the aim of assessing the sustainability aspects of low-carbon hydrogen in the EU. A significant proportion of the reviewed articles were concerned with LCA performance, either as a standalone method or in conjunction with other approaches. As the LCA is mostly focused on the environmental dimension, alternative methodologies such as the Technoeconomic Assessment or Life Cycle Cost were utilized complementarily to address the technical and financial evaluation of the projects. Furthermore, Multicriteria Decision-making Methods were employed, incorporating multiple aspects, along with other relevant assessment approaches.

The present study constitutes an exhaustive analysis of the methodologies employed in conjunction with LCA to address the sustainability of low-carbon hydrogen projects. LCA is a frequently used approach for the evaluation of low-carbon hydrogen with a focus on its environmental dimension. Nevertheless, in order to perform a comprehensive assessment of low-hydrogen initiatives, it is imperative to integrate LCA with alternative approaches.

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5.04.P Complementing and Combining LCA with Other Methodologies for Better Decision Making

5.04.P-We361 Recommend Economic, Environmental and Social Consideration for Cereal Production socio-eco-efficiency evaluation and predictive optimisation

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Sustainable development has been the interest of multiple researchers and scholars worldwide for centuries. A modelling approach to orient the world for evaluation from the socio-eco-efficiency perspective is vital to empower the collaborative efforts and innovative approaches aiming to build a resilient and inclusive agricultural system meeting current demands, preserving ecosystems and supporting future generations. The conducted study aims to develop, particularly in the era of European cereal production, a holistic optimising model, which considers various dimensions and factors to catalyse positive change and pave the way for a prosperous and sustainable future in light of industrial ecology and circular economy methodology, tools, and principals. Results of this study have shown that all investigated countries were able to achieve a high value of the “Sustainability index” indicator in the last years. However, an analysis carried out comparing all the input variables has concluded the necessity to act on the main variables related to Climate Change, Ozone, Photochemical Oxidation, Abiotic Depletion, and Toxicity to attain the ideal scores equal to 1,00. Accordingly, the developed model could help users, policymakers, scientists, scholars, governments, and communities not only in Europe, but also worldwide to frame their knowledge for achieving the sustainability goals and to orient them per aspect, per family, and per category for better categorisation and multi-dimensional speciality to find a suitable strategy and to establish the right rule in the micro, and macro environment, from the operational level to the strategic one.

5.04.P-We362 Challenges of Social Life Cycle Assessment in Agri-food systems

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Environmental Life Cycle Assessment (eLCA) is the reference method to evaluate the environmental impacts of products throughout their life cycle. Yet there is a growing need for methods integrating more sustainability dimensions into decision making. Social Life Cycle Assessment (S-LCA) has emerged in this context for the assessment of social impacts across the value chain of products. Despite its potential, the application of S-LCA in agri-food systems remains underdeveloped, particularly in the context of accounting for the background through databases.

This study aimed to identify key challenges specific to the application of S-LCA to the agri-food sector and how database integration can enhance the robustness of such social impact evaluations. We first conducted a scoping review to identify challenges in S-LCA applications and then applied S-LCA to conventional apple and tomato production in Switzerland. The case studies followed the S-LCA guidelines and used the PSILCA v3 database to account for background processes, allowing a direct comparison with eLCA.

We classified the challenges into the ones related to (1) boundary conditions, (2) S-LCA steps, and (3) the integration of S-LCA with other sustainability assessment frameworks. Specific challenges for agri-food systems include the underrepresentation of animals in S-LCA and the lack of a stakeholder category and indicators accounting for the specificities of farmers. Additionally, the granularity of data in existing S-LCA databases does not align well with that of eLCA, limiting the comparability of both approaches.

The case studies revealed that high-value input processes—such as worker wages and trade union density— influence the social risks the most. However, the use of non-specific processes for Switzerland questioned the representativeness of the results. Still, the social hotspots identified stayed robust to the modelling assumptions.

To advance the applicability of S-LCA in agri-food systems and increase its usefulness in decision-making processes in combination with eLCA, future research should focus on integrating agricultural-specific flows in existing databases, improving the representation of farmers and animals, and enhancing the alignment of social and environmental LCA naming. This research contributes to integrating life cycle thinking with innovative frameworks to address the complex interactions between the environmental, economic, and societal dimensions of sustainability.

5.04.P-We363 Forecast and Prevent: A dynamic LCA-integrated Decision Framework for Reducing Food Waste in the Visitor Economy

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Background: In the tourism and hospitality sector, procurement logistics related to food acquisition patterns contribute significantly to greenhouse gas emissions and resource consumption, primarily driven by the need to maintain high service levels. Yet, decisions on ordering, redistribution and menu design are rarely guided by integrated environmental and operations modelling.

Purpose: The project develops a decision framework that couples supply chain modelling, demand and surplus forecasting, and life cycle assessment (LCA) for perishable food chains in a coastal tourism region. Rather than focusing only on model performance, the project is explicitly oriented towards providing actionable information to retailers, hospitality operators, and public authorities.

Approach: The analysis examines two supply chain networks, including dairy products and beverages, focusing on order and return policies, ingredient reallocation, and waste reduction strategies. System dynamics models are created to demonstrate food flows in the tourism and hospitality sectors from production to consumption or recovery, along with key influencing variables. Additional data layers, such as inventory and booking information, offer critical context for product streams, with booking patterns significantly affecting order values. LCA models are integrated into these flows to dynamically assess the climate, water, and land use impacts of various logistics and reverse logistics policies. By continuously combining these models with managerial decisions, the framework captures an evolving LCA perspective rather than a static snapshot.

Relevance: The framework helps the industry identify strategies that reduce waste and emissions while keeping service levels and margins intact. It illustrates trade-offs between prevention and end-of-pipe solutions. For policymakers, it offers a way to measure the environmental gains of prevention-focused approaches, aids in designing incentives and reporting standards, and provides a transparent tool to align food waste reduction, climate goals, and social objectives at the destination level. The presentation will demonstrate, through an initial pilot, how this integrated framework can inform decisions at both corporate and regional levels.

5.04.P-We364 Can You Keep (Your) Score? How Methodological Choices Affect the Sustainability Score of Your Food

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Literature has highlighted the need for a harmonised methodology for assessing the sustainability of food products. In this context, we developed a concept for scoring the sustainability of food products, combining both LCA and non-LCA elements across all dimensions of sustainability (environment, economy, society, animal welfare, and possibly even health).

A wide range of methodological decisions defines the resulting calculation and assessment tool. An in-house expert consultation has led to a first set of methodological choices, which need to be validated through extensive stakeholder processes and sensitivity analyses. While the LCA part is currently based on the PEF single score of a food product, the non-LCA elements incorporate information such as the presence of certification labels (such as those referring to organic farming or a higher animal welfare standard) or the implementation of biodiversity-enhancing measures at the farm. The LCA and non-LCA-elements are combined using distinct weighting factors. The resulting sustainability score then rates food products on a [0,100] scale and can be applied to both single as well as composite products.

This poster sheds light on how methodological decisions affect the score obtained for a selection of products. Focus hereby lies on: weighting assigned to the LCA score; weighting assigned to the non-LCA elements; indicators representing the non-LCA elements; choice options for each non-LCA indicator and values assigned to them; and finally, rating approach in terms of number of classes and setting of thresholds. All of these decisions need to be finalised in co-creation with food system actors. The examples shown on the poster highlight the importance of harmonisation and transparency to avoid cherry picking by those applying an assessment scheme and avoid confusion by consumers who might want to base their purchasing decisions on such scores.

Disclaimer/Disclosure:

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5.04.P-We365 A Combined Sustainability Approach to Guide Infrastructure Design for Poly(hydroxyalkanoate) Production from Food Waste

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The shift from fossil-based polymers and the growing focus on circular bioeconomy strategies have increased the need for bioprocesses capable of synthesizing sustainable biopolymers. Poly(hydroxyalkanoate)s (PHAs) are promising microbial polyesters, yet its production from virgin substrates raises costs and pressure the food supply chain. Using carbon-rich organic residues as alternative feedstocks can lower environmental and economic burdens while addressing waste-disposal challenges. However, the sustainability of this route depends heavily on the design and operation of the supporting biorefinery system. The BioLaMer project investigates a black soldier fly larvae (BSFL) biorefinery converting food waste (FW) into high-value biopolymers, requiring strategic decisions on process configuration before scaling up.

This study addresses the challenge of evaluating the potential environmental impacts of alternative production strategies for cultivating BSFL fed with FW to produce 1 ton of PHA per year. The study shows how early-stage Life Cycle Assessment (LCA) can provide valuable insights for defining the business model of an innovative production system, by highlighting environmental trade-offs between two alternative configurations: (i) a decentralised network of BSFL cultivation plants, located near FW sources within the urban area; and (ii) a centralised setup. While decentralisation may help reduce FW transport, it may also introduce challenges such as more frequent maintenance, distributed energy consumption, and additional logistics for larvae collection. This analysis uses the scale-up data estimated for Life Cycle Costing (LCC) based on the productivities achieved at laboratory scale so far (~10 kg of wet larval biomass per each larvae farm with 35-d cycle growth). Such upscaled data are translated into material and energy flows for a gate-to-gate LCA using the Environmental Footprint 3.1 method, focusing on environmental hotspots and key contributors across both scenarios.

Preliminary sustainability assessment has already been guiding the biorefinery design, with an energy consumption analysis to find solutions to accommodate lower temperatures during winter season. In conclusion, the study assesses whether combining LCC-based upscaling with early-stage LCA can show how sustainability assessment supports system design before scale-up.

Disclaimer/Disclosure:

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5.04.P-We367 Visualizing multi-way relationship of environmental burdens and economic benefits in global supply chains of nations

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This study develops an analytical framework to visualize the multi-way relationships among environmental burden footprints and socio-economic benefits while capturing their propagation structures along global supply chains (GSCs) of industries of nations. Specifically, using the multi-regional input-output model, we estimated GHG emissions and freshwater consumption footprints and aggregated embodied intensities (AEIs), defined as burden footprints per unit of PPP-based value added, at each propagation tier of the relevant GSCs, which implicate the eco-efficiency of the GSCs. Results showed the structures of the relevant burden footprints along the propagation tiers of the GSCs with their eco-efficiency. For example, in the case of manufacturing GSCs in the USA, the freshwater footprint is concentrated at tiers close to final production, and the corresponding AEIs exceed the global average at almost all tiers, while those of GHG emissions were below the average, indicating relatively high urgency of efforts to reduce the freshwater footprint especially at closer tiers of the relevant GSCs in terms of enhancing the eco-efficiency of the GSCs. We applied the proposed framework to GSCs of all

industries of nations and identified the key features for footprint reduction and eco-efficiency enhancement in the relevant GSCs.

5.04.P-We368 A Framework for Evaluating Nutrients Recovery Alternatives

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Nutrients recovery from source-separated organic fraction of municipal solid waste (SS-OFMSW) is a waste valorization process, and it is becoming an integral part of a waste management system, since it not only reduces the loss of natural resources but also upsurges the production of value-added bio-products. There are a number of treatment alternatives for nutrients recovery from organic waste, characterized by different social, economic, environmental, and technical performances. However, the identification of the most suitable alternative from multiple alternatives becomes difficult for a decision-maker due to the complexity of life cycle-based methodologies and data requirements. Under this light, the objective of this study is to show how to select the most suitable nutrients recovery treatment alternatives among multiple alternatives where data availability is limited.

To meet the objective, this research is developed through two steps. First, this study analyzes 31 different studies on LCA of waste management and treatment alternatives for nutrients recovery from municipal solid waste (MSW) and explores the most impacting phases based on types of input wastes and recovered nutrients, functional unit, processes included within the system boundaries, allocation procedures, and applied life cycle impact assessment (LCIA) method. Secondly, to assess the social, economic, environmental, and technical performance of treatment alternatives by a single effort, the integration of multi-criteria decision analysis (MCDA) with LCA is examined.

This research summarizes the most impactful phases of environmental impact for different nutrients recovery alternatives, facilitating the identification of the most suitable nutrients recovery alternatives. Finally, this study proposes an integrated framework of LCA and MCDA for evaluating the sustainability performance of nutrients recovery treatment alternatives.

Disclaimer/Disclosure:

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5.04.P-We369 Material Flow Analysis and Life Cycle Assessment of Aluminium: Challenges and Steps Toward Circularity

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Aluminium is a crucial material in the EU's industrial system, particularly in the transportation, construction, and packaging sectors. As a Critical Raw Material (CRM) and Strategic Raw Material (SRM), aluminium poses risks to the EU's resilience and competitiveness. Among all SRMs, aluminium consumption has the most significant environmental impact in the EU, due to its high demand in various industries. To mitigate these risks, unlocking the full potential of aluminium recycling and closed-loop production is essential. This study will present the state-of-the-art of combining dynamic Material Flow Analysis (MFA) and Life Cycle Assessment (LCA) to the aluminium value chain. By integrating dynamic MFA with LCA, the study provides a framework for assessing the environmental impacts of the aluminium value chain and promote sustainable practices. The findings will reveal substantial environmental impacts caused by the EU's resource and trade dependency on third countries, and will underscore the importance of considering global dynamics in assessing environmental impacts. This study will leverage insights on key areas: environmental impacts, secondary material, stock dynamics, end-of-life management, and alloy complexity. Handling the complexity of the aluminium value chain could pave the way forward for substantial advancements in sustainable aluminium production and lifecycle management. The importance of advanced sorting processes for optimizing aluminium scrap treatment and understanding distinct types of aluminium process losses will be highlighted. As well as the need to address alloy differentiation and the potential for downcycling during recycling, which can affect the environmental benefits of secondary production. Overall, these advancements are crucial for strengthening the EU's resilience

and competitiveness while reducing the material's criticality and environmental footprint associated with the aluminium value chain, thereby supporting a more circular and sustainable economy.

5.04.P-We370 Life Cycle Assessment of Buildings Based on Forest Carbon Sequestration Using Satellite Data

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In order to achieve a carbon-neutral society by 2050, the building sector—which accounts for roughly 30% of total energy consumption—must urgently accelerate decarbonization through the use of wood and other low-impact materials. Wood has recently attracted attention as a key means of reducing the carbon footprint of buildings, not only because it requires less energy during production than steel or concrete, but also because trees absorb CO₂ during their growth and store it as carbon within their biomass, which can then remain fixed in buildings over long periods. Through this carbon-sequestration effect, the long-term and widespread use of wood enables a continuous and sustainable carbon cycle from forests to buildings, thereby increasing carbon stocks across society and contributing to broader climate-change mitigation efforts.

Against this background, Japan is scheduled to enact the Building Environmental Performance Assessment Act (“Building LCA Act”) in 2028, which will mandate comprehensive environmental impact assessments for buildings. Under this legislation, environmental burdens must be quantified across the entire life cycle of buildings, making the treatment of carbon stored in wood a critical and often debated issue. Carbon sequestration varies significantly depending on factors such as tree species, age, forest management practices, soil conditions, and climate, meaning that precise evaluation based on the wood's procurement region is essential for credible and scientifically sound assessments.

This study focuses on differences in the harvesting regions that supply wood and seeks to capture spatiotemporal variations in forest carbon sequestration by considering carbon stored in trees and soils, as well as carbon dynamics associated with land-cover change, using satellite-derived data and geospatial analysis. The resulting forest information is then integrated into building LCA to quantitatively assess the environmental impact-reduction effects of wood use in buildings under different sourcing scenarios. Through this approach, the study aims to clarify how regional differences—including species composition, forest management, and land-use modifications—are reflected in the life-cycle environmental impacts of buildings, thereby providing insights that may support more sustainable material procurement and informed policy-making.

5.04.P-We371 Where to install the next direct air capture and storage plant? Optimal Technology Deployment via Integrated Lifecycle Assessments and Techno-Economic Optimization

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Carbon Dioxide Removal (CDR) solutions are indispensable for achieving global net-zero climate goals. A promising CDR technology is Direct Air Carbon Capture, Transport and Storage (DACCTS). As DACCTS moves toward commercialization, the need for integrated assessment methodologies is critical to provide ex-ante assessments of CDR efficiency, life-cycle environmental performance, and costs. Due to the anticipated gigaton-scale deployment, we must ensure climate benefits across varying regional and temporal conditions. Here, we develop a holistic and integrated framework to optimize DACCTS system performance and costs across regions and time. The bottom-up assessment combines geospatial modeling, energy-system analysis, spatially- and temporally-explicit techno-economic optimization of solid sorbent DACCTS configurations, CO₂ transport-routing analysis, and life-cycle assessment (LCA). The framework captures regional and temporal climate variability impacting the specific energy demand, as well as renewable and grid electricity availability, land constraints, CO₂ transport and storage logistics, and technology-specific learning rates. We examine grid-connected, off-grid, and hybrid configurations for 2025 and 2050 across multiple climate pathways.

Two main methodological innovations distinguish this work: First, future climate conditions and weather extremes are incorporated through different climate scenarios to identify regions where DACCTS deployment remains resilient to evolving climatic stresses. Second, LCA is applied to the region- and time-specific DACCTS supply chain, with optimal CO₂ transport routes determined based on locally available multimodal transport infrastructure and storage sites. Together, these elements identify favorable climate, energy, and economic conditions that justify siting DAC plants farther from storage sites, and where the development of new storage hubs would most substantially reduce system-wide costs and impacts.

By coupling LCA with spatial-temporal techno-economic optimization, the study identifies optimal regions for

DACCTS deployment, i.e., cost-effective, low-impact locations where CDR can be achieved at scale. The results demonstrate the benefits of integrating LCA into a multi-method decision framework by providing actionable insights for policymakers, investors, and industry that pinpoint resilient, cost-effective, and environmentally robust regions for DACCTS development.

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5.04.P-We372 Circularity and Life Cycle Assessment of climbing holds made from recycled polyamide climbing ropes

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With growing demand for sustainable, circular practices, the sports industry is under pressure to adopt circular economy principles and reduce its environmental impact. However, little is known about how circular design strategies affect environmental and circularity performance in sports equipment. Climbing holds are commonly manufactured from non-recyclable thermoset resins, generating significant environmental burdens and limited circularity. This study addresses the environmental impact and circularity of redesigned climbing holds through Design from Recycling, Design for Recycling, and Design for Durability.

Replacing thermosets with mechanically recycled polyamide 6 (PA6) reduced environmental impacts by 90% across key categories, including climate change, marine ecotoxicity, fossil resource depletion, and human toxicity. Short-loop recycling from climbing ropes showed lower environmental impact compared with long-loop recycling from automotive waste. Circularity scores align with environmental performance, with virgin thermosets below 0.1 and recycled PA6 scenarios above 0.9. Lifetime extension for recycled PA6 holds further reduced inflow energy demand, although these benefits were only partially captured by Material Circularity Indicator (MCI) values. Including mould use increases the impact of thermosets, as silicone casting moulds have a short lifetime. For rPA6, durable aluminium injection moulds reduced the impact per hold.

These results demonstrate that short-loop recycling of climbing ropes into climbing holds is a viable and scalable circular strategy. Next, combining Design from Recycling, Design for Recycling, and Design for Durability significantly reduces environmental burden. Additionally, combining Life Cycle Assessment (LCA) with circularity indicators provides a complementary perspective. LCA captures the scale of environmental benefits, whereas circularity metrics highlight structural improvements. These results show that circular strategies can support a more sustainable product design in the sports equipment sector.

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5.04.P-We373 Integrating Life Cycle and Circularity Assessment to Guide Eco-design of Functional Electronics

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The transition to sustainable and circular electronics requires decision-making that extends beyond traditional product performance indicators. Within the UNICORN project, Life Cycle Assessment (LCA) and Circularity Assessment (CA) were combined to identify, evaluate, and prioritise eco-design measures for functional electronics integrated into smart systems.

Four use cases, a smart battery casing, a dashboard module, a sensing system with integrated electronics and a tire sensor, were analysed from a cradle-to-gate perspective, using inventory data from industrial partners and theecoinvent database. Environmental impacts were assessed using the European Commission's Environmental

Footprint (EF) method, covering 16 midpoint indicators such as climate change, acidification, eutrophication, and resource use. Circularity performance was evaluated using a set of indicators adopted from ISO 59020 that include, among others, renewable, recycled, and reusable material inflows and outflows. This integrated approach enabled the identification of design improvements that simultaneously reduce environmental impacts and enhance circularity.

Results show that targeted material optimization, such as the use of recycled metals, can substantially improve environmental and circularity performance when implemented early in the design stage. However, trade-offs must be carefully assessed, as environmental gains in terms of climate change may be offset by increases in other impact categories, such as land or water use, or by heightened reliance on critical raw materials, or reduced recyclability due to altered material chemistry. For example, the use of alternative metals like copper in conductive inks in the dashboard module lowered the contribution to ecotoxicity, but at the same time the use of critical materials increased.

The integrated use of LCA and CA provides a structured, evidence-based approach to support decision-making in product development. By identifying the most promising design levers, such as the application of more environmentally friendly conductive inks, and exposing potential trade-offs early in the innovation process, this combined methodology enables more balanced, system-level optimisation.

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5.04.P-We374 Refined Methodology for Assessing Corporate Water Footprint Across the Entire Value Chain

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Water is an indispensable resource, contributing significantly to global economic value. For businesses, understanding how water is utilized across their value chain is crucial for managing operational risks, minimizing potential profit losses, and ensuring the sustainable use of this resource.

The cosmetics industry relies on water as a key component in the production of its raw materials, manufacturing, and usage of its products. Water footprint assessments have focused primarily on blue water (surface and ground water) and grey water (wastewater), frequently overlooking the role of green water (rainwater) and the interconnection of hydrological cycles.

The primary objective of the methodology is to provide a detailed and data-driven analysis of the impacts and dependencies on water across L'Oréal's value chain, using the latest available data and enabling a more precise modeling of its impact on both water quantity and quality. The updated approach refines the previously followed water footprint methodology by incorporating green water volumes, thereby achieving a more comprehensive understanding of water dependencies. The methodology also distinguishes between direct and indirect impacts, offering clearer insights into where levers can most effectively be activated. The assessment covers the full value chain, from raw material production to finished product end-of-life, and utilizes indicators such as water withdrawals, freshwater eutrophication, and freshwater ecotoxicity.

This assessment provides enhanced insights into the water impacts of purchased goods, distinguishing their influence on withdrawals and freshwater eutrophication. It also features refined crop water needs modeling, incorporating regional requirements and green water contributions to understand primary water sources. Furthermore, it details impacts from finished product use and end-of-life on freshwater ecotoxicity and rinsing-related water withdrawals.

Just like a refined carbon footprint, this analysis becomes an internal decision-making tool for managing water-related risks and guiding action plans.

5.04.P-We375 From lab to rail: A Combined Mechanical LCA Matrix for Fiber Reinforced Polymers

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The design of next generation vehicle structures increasingly demands novel material systems that combine high mechanical performance with environmental sustainability. While mechanical criteria traditionally dominate material selection, developments in sustainability requirements have exposed a gap in current multi criteria decision making (MCDM) tools. In this regard most frameworks focus only on climate related life cycle impacts, neglecting other significant environmental impact categories. This study presents a selection matrix tailored for fiber reinforced polymer (FRP) systems that explicitly integrates all impact categories of the environmental footprint method at the early design stage.

Initially, prevailing MCDM approaches are screened and consolidated to identify common decision criteria, weighting schemes, and data sources. Based on these results, a set of application specific FRP constituents are chosen, encompassing various polymer matrices and reinforcing fibers. Mechanical descriptors can be collated from literature and mechanical testing, while ecological characteristics were derived from life cycle inventories (LCI) customized for each polymer/fiber pair. Additional LCIs were assembled using the structure of established databases but modified to capture the heterogeneity of polymer systems.

The resulting framework was validated first on individual materials and subsequently on material combinations. Each combination is classified within the matrix reflecting the mechanical and environmental performance. Comparative analyses highlight possibilities, revealing that materials that perform better from a mechanical standpoint can suffer substantial impacts in categories such as ecotoxicity or resource use. Conversely, polymers with lower mechanical properties can achieve better results regarding potential environmental impacts when coupled with appropriate fibers. It is therefore essential to consider both sides simultaneously.

The simplified matrix offers practitioners an intuitive, data driven tool that can be applied early in the design process, thereby enabling concurrent assessment of performance and sustainability. By bridging the methodological divide between mechanical engineers and LCA experts, the matrix facilitates interdisciplinary collaboration and supports evidence based material decision making for future structural elements.

5.04.P-We376 Thermal-Comfort Impacts of Residential Heat Pumps

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Ensuring efficient and sustainable heating and cooling is essential for the energy transition, yet thermal comfort depends on the interplay among building insulation, heating technologies, occupant behavior, and policy and infrastructure conditions. Heat pumps are particularly relevant due to their dual heating and cooling functionality, compatibility with renewable electricity, and technological maturity. However, despite their promise, important questions remain regarding the most sustainable pathways forward, as previous life cycle assessment (LCA) studies often simplify behavioral influences and ignore system-level feedbacks.

This work presents a system dynamics (SD)-guided parametric LCA framework combined with global sensitivity screening to investigate the causal factors shaping the environmental impacts of heat-pump-based thermal comfort. The SD model represents interactions among building characteristics, climate, comfort preferences, and policy-driven changes in electricity carbon intensity. Because electricity consumption during operation is consistently identified as the dominant environmental hotspot, the SD model focuses on the adoption and use phases of residential heat pumps. This structure enables a high-dimensional, feedback-aware exploration of how uncertainties and nonlinear relationships propagate through environmental impacts. The integrated SD-LCA approach investigates which technological, behavioral, and policy factors most strongly influence the sustainability of heat-pump heating and cooling, helping to identify interventions with the highest leverage. The study establishes a foundation for future work that further advances dynamic, system-oriented methods in sustainability assessment.

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5.04.P-We377 Comparing Simplified Environmental Indicators with LCA: A Case Study on Noble Metals Recovery from E-Waste

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The application of simplified sustainability assessment tools during process design can be particularly helpful for the comparison of alternatives and the early adoption of more environmentally efficient solutions. This is especially relevant for processes aimed at recovering critical raw materials from waste. The ESCAPE approach (Evaluation of Sustainability of material substitution using CARbon footPrint by a simplifiEd approach) was developed to provide a simplified sustainability index based on carbon footprint and embodied energy, allowing rapid comparisons between recovered or substitute materials and their primary counterparts. In this study, we applied both the ESCAPE approach and the Life Cycle Assessment (LCA) methodology to a hydrometallurgical method designed to recover copper, silver, and gold from waste Random Access Memory (RAM) modules. The process relies on benign, selective, and recyclable reagents under mild conditions, and is designed to offer a more sustainable alternative to conventional hydrometallurgical recovery routes. This study aimed to assess the consistency between ESCAPE and LCA in identifying main environmental impact contributions. Additionally, results were used to perform a preliminary benchmark of the sustainability of the recovery method against conventional primary extraction processes for noble metals, based on available industrial data. Overall, the ESCAPE approach effectively identified a significant portion of the system's potential environmental impacts, closely aligning with LCA findings. However, discrepancies emerged in specific impact categories and when applying economic allocation within the LCA. Different LCA end-of-life modeling approaches were also examined to better understand how they affected the results. Based on these findings, we highlight strengths and limitations of the two approaches to define the most appropriate application boundaries for their effective use. We also propose a dual-assessment framework integrating ESCAPE and LCA to promote the adoption of sustainable practices particularly during the early-stage experimental design.

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5.04.P-We378 Integrating persistent mobile toxic chemicals into life cycle assessment: a cradle-to-grave evaluation of a low-carbon, nature-based wastewater treatment technology

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Persistent, mobile, and toxic chemicals (PMTs) are widespread in aquatic environments, with wastewater as their primary source. As freshwater scarcity intensifies, wastewater reuse is critical for sustainable water management. However, traditional wastewater treatment processes are largely ineffective at removing PMTs. Advanced oxidation and chemical treatments, also known as tertiary treatments, can be effective, but are energy-intensive and may generate secondary pollution products. With their higher sustainability, nature-based solutions offer a promising alternative to mechano-chemical tertiary treatments, but they typically have a large land-use footprint and slow treatment rates limiting their scalability and economic viability. A recently developed nature-based solution leverages the filter-feeding capabilities of *Daphnia* (waterfleas) to capture and bioaccumulate a broad spectrum of PMTs, along with excess nutrients and suspended solids. As an emerging technology, its life cycle environmental impacts remain unassessed. This study presents the first cradle-to-grave life cycle assessment (LCA) of this emerging technology. Unlike conventional LCAs, our study bridges the gap between chemical water quality assessment and life cycle thinking, integrating contaminants typically overlooked in standard LCA frameworks. Results show that the technology achieves a low overall environmental footprint, while reducing ecotoxicity and eutrophication potential. Scenario-based analysis indicates that recycling of infrastructure materials can reduce resource depletion, further reducing the carbon footprint of the technology. This LCA sets a new benchmark for evaluating wastewater treatment technologies and highlights the strong potential of the *Daphnia*-based technology as a sustainable, scalable option for tertiary water treatment and reuse.

5.04.P-We379 Integrating the Impacts of Wildfire into Life Cycle Assessment: An Approach Applied to an Extensive Livestock System

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Global change is amplifying challenges in landscape management, particularly through the increasing frequency and severity of wildfires, which cause substantial losses in lives, biodiversity, and ecosystem services (ES). Climate change is expected to exacerbate this trend, while socioeconomic drivers such as rural depopulation and land abandonment further heighten wildfire risk by altering land use and increasing fuel loads. In Europe, abandoned rural areas are recognized as wildfire hotspots, making fuel management a key priority. Wildfire protection itself is an ES that can be compromised by human activities, yet current Life Cycle Assessment (LCA) approaches do not account for wildfire protection. This study addresses this gap by proposing a novel method to integrate wildfire impacts into LCA. Specifically, we (1) identify impact pathways linking environmental interventions in the Life Cycle Inventory (LCI) related to fuel-load management with a new wildfire protection impact category and existing indicators, and (2) test the approach in a case study comparing wildfire-related impacts with and without fuel management through grazing. A literature review was conducted to establish relevant causality chains, operationalized using fire behavior modeling (FlamMap) and characterization factors (CFs) for impact categories. First, changes in biomass load are quantified and translated into fire intensity, efficiency, and emission factors (e.g., CO₂, PM₁₀, SO₂), which vary with fuel type and moisture content. These emissions are then multiplied by CFs to generate category-specific impact scores. The method is applied to an extensive, multifunctional small ruminant herd in the Spanish Pyrenees, comparing scenarios with and without livestock presence. Results link wildfire impacts to existing LCA categories such as climate change, particulate matter, and acidification, while highlighting data gaps for others (e.g., eutrophication, ecotoxicity). Unlike conventional LCA, this approach addresses an environmental hazard typically assessed through risk-based methods, revealing the potential benefits of extensive livestock systems often deemed less eco-efficient than intensive ones. By incorporating wildfire impacts, this framework supports more holistic sustainability assessments and informs land management, food security, and climate adaptation policies in vulnerable regions.

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5.04.P-We380 Variability of Logistics Services: Insights for Life Cycle Assessment

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Logistics decarbonization has emerged as a new stream for extended sustainable operations, reducing the impacts by designing logistics solutions with lower CO₂ emissions. However, capturing the logistics emissions across supply chains still represents a challenge fed by the difficulty in knowing the practices along the supply chain and how to measure them.

In light of this, this research aims to reveal how to capture the dimensions of logistics service variability to improve life cycle inventory modelling. Our research focuses on developing a life cycle inventory (LCI) capable of reflecting the inherent complexity and high variability of logistics services. To achieve this, we adopted a mixed data collection approach combining both qualitative and quantitative data. Specifically, we engaged multiple logistics service providers (LSPs) and requested detailed information regarding their operations performed for the same company. The inquiry was structured around two main operational domains: warehouse management and transportation activities. By comparing how different LSPs manage storage, energy use, vehicle operations, and transport planning for an identical customer, we captured the heterogeneity of service provision and identified representative parameters for inventory modeling.

One set of variations concerns warehouse management, including energy consumption, the use of other energy carriers, water usage, space utilization, and stock management. Another set of variations relates to transportation practices. In addition to the fuel types and vehicle categories already commonly considered in LCA studies, further variability was identified in route management, driving habits, driver-assistance measures in traffic conditions, vehicle load factors, loading/unloading cycles, and the co-functionality of services provided to different customers.

The analysis highlights the significant variability that characterizes logistics services, both in warehouse operations and transportation practices. Understanding these differences is essential for designing more accurate life cycle inventories and supporting the development of more efficient decarbonization solutions for logistics.

5.04.P-We381 Modeling Ecolabel Certification Credibility under Climate Uncertainty: Toward Integrated Sustainability Assessment

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Ecolabels play a key role in promoting sustainable resource management, yet their reliability under climate uncertainty remains insufficiently understood. This study addresses the challenge of how environmental variability affects the credibility and monitoring needs of fishery certification schemes.

A game-theoretic signaling framework is developed to analyze the strategic interaction between fishermen seeking certification and a monitoring authority operating under incomplete information. The model identifies the conditions under which certification credibly conveys environmental performance (separating equilibrium), and the conditions under which it fails to do so (pooling equilibrium). The model incorporates certification costs, expected profit gains, and the probability of unfavorable environmental conditions.

The results show that moderate certification costs, asymmetric economic gains, and higher environmental risk promote informative certification, thereby reducing the need for direct monitoring. Conversely, low certification costs or generous subsidies can erode its credibility, increasing oversight requirements.

These findings offer quantitative insights for integrated assessment and sustainability governance, demonstrating how institutional and behavioral uncertainty can complement traditional Life Cycle Assessment (LCA) approaches. This framework supports the design of adaptive, risk-informed certification systems, advancing life cycle thinking in policy and natural resource management.

5.05.P Embracing Outliers Within the Emerging Nexus of Agriculture, Food Systems, and the Bioeconomy

5.05.P-Tu421 Bio-based Fertilisers from Fisheries Wastes: An Environmentally Friendly Alternative to Mineral Fertilisers?

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The production of mineral fertilisers poses several sustainability issues, including high energy use (nitrogen fertiliser) or resource depletion (phosphorous fertiliser). To address these challenges and related geopolitical dependencies, the nutrient recovery from biowaste streams in the form of bio-based fertilisers (BBFs) is increasingly promoted. There is a growing number of life cycle assessment (LCA) studies on BBF production from common biowaste streams such as sewage sludge or animal manure. Despite fisheries waste being another abundant and nutrient-rich side stream, information on the environmental impacts of its transformation to BBFs and their application is limited. This study aims to address this lack of information by assessing environmental impacts of three BBFs produced from fisheries waste and their application in comparison to mineral fertilisers.

The system boundary included the transport of raw wastes, their transformation to BBFs, the transport to the farm and their application in broccoli cultivation. The functional unit considered was 1 kg of broccoli.

Foreground data on three BBFs was collected from two pilot-scale and one industrial-scale production plants in Europe. Information on the application of these BBFs was based on a field trial with broccoli in Estonia. The assessment was conducted with the FarmLCA tool and Brightway 2.5 and included five midpoint indicators: Climate change (GWP100), Terrestrial acidification, Marine eutrophication, Freshwater eutrophication and Mineral resources use.

Regarding Global warming and Mineral resources use, two of the BBFs had similar or lower impacts compared to mineral fertiliser (1-13% lower). For Terrestrial acidification, Marine eutrophication, and Freshwater eutrophication, the mineral fertiliser showed the lowest impacts. While the production of BBFs contributed up to 49% and 54% to the impacts of Global warming and Mineral resources use, respectively, field emissions dominated the other three impact categories. Yet, sensitivity analyses also revealed uncertainties, e.g. for the modelling of NH₃ emissions.

The analysis has shown that the production of BBFs and their agronomic performance should be further optimised to serve as environmentally friendly alternative to mineral fertilisers. This study provides guidance for such an optimisation process by identifying relevant contributions and levers during production and application of BBFs.

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5.05.P-Tu422 Quantification of the Relevance of Time-Dynamic Carbon Accounting for Different Feedstock and End-of-Life Emission Scenarios of PLA

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As a biobased polymer with the possibility to degrade in a reasonable period of time, Polylactic Acid (PLA) is often regarded as a sustainable alternative to fossil-derived plastics and a leading polymer in the bioplastics sector. Recent studies show that the impact of biogenic carbon on the global warming potential is subject to the temporal dynamics of biomass growth rates and end-of-life (EoL) processes. However, current LCAs often apply a static approach when assessing biogenic carbon. Thus, such a static assessment overlooks the impact of temporary carbon storage and neglects that carbon neutrality does not equal climate neutrality. Consequently, the climate impact of PLA is not yet well depicted in current assessments.

The present study addresses this challenge by quantifying temporal carbon dynamics through the application of a Biobased Materials Life Cycle Assessment (BBM-LCA) framework and comparing the impacts of different feedstocks and EoL treatments on the climate impact of PLA. Our approach integrates time-dependent life cycle inventories, net ecosystem exchange (to represent carbon uptake and release during feedstock cultivation), and dynamic characterization factors. We evaluate how different feedstocks (corn, sugarcane, and sugar beet) and EoL pathways (mechanical recycling, chemical recycling, composting, and incineration) shape the timing and magnitude of emissions, and how temporal dynamics alter conclusions on the overall impact compared to a conventional static assessment.

Preliminary results show that the static approach overestimates the global warming potential compared to dynamic accounting by up to 56%. Although EoL emissions represent only a small share of total emissions, accounting for the timing of biogenic carbon release leads to significant differences: the dynamic LCA indicates a lower GWP100 by 45% for delayed emissions for the composting scenario of PLA and a 56% reduction for chemical recycling.

Our findings underline that a dynamic perspective on biogenic carbon cycles materially shifts the environmental profile of biobased plastics and thus must not be neglected for a comprehensive sustainability assessment of PLA.

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5.05.P-Tu423 Decoupling Biodiversity Footprints of Food Consumption from Economic Growth Varies with the Wealth of a Country

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Food systems pose a critical threat to global biodiversity, yet it remains unclear whether and under what circumstances biodiversity footprints can be decoupled from economic growth, which is essential for sustainable development. Here we quantify changes in land use-based biodiversity footprints of food consumption in 158 countries (1995 - 2020) to evaluate whether country-specific changes in the biodiversity footprints of food consumption are (de)coupled from economic growth. We used the Food and Agriculture Biomass Input-Output

(FABIO) multi-regional input-output (MRIO) model to derive these land use-based biodiversity footprints. We adopt a consumption-based biodiversity footprint approach, which accounts for both domestic and transboundary impacts to explore decoupling. We consider two commonly used and complementary biodiversity indicators to quantify biodiversity losses associated with food consumption: the potentially disappeared fraction of species, i.e., the relative loss in global species richness (RSR-loss); and the decline in local ecosystem intactness, i.e., the loss of mean species abundance (MSA-loss). RSR-loss measures the loss in species richness relative to a natural reference condition. MSA-loss reflects the average loss in species abundance relative to a natural reference condition (while not considering increase in species abundance). We find a strong decoupling in 8-9%, weak decoupling in 62-70%, and coupling in 22-29% of the countries. Decoupling is more likely at higher GDP per capita levels and more evident for plant-based food consumption compared to animal-based food consumption. However, total biodiversity footprints remain dominated by animal-based food consumption, constraining the potential for global decoupling. These results show current levels of decoupling are not yet sufficient to achieve global net reductions in biodiversity footprints.

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5.05.P-Tu424 Mapping Biomass Flows for Material Use in Switzerland: Towards a Systemic Framework for Biomass Valorisation

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A systemic understanding of how biomass is produced, processed, and utilised within the technosphere and society is necessary to understand the sustainable biobased transition of materials. Biomass plays a crucial role in achieving net-zero goals by substituting fossil-based materials and storing biogenic carbon in long-lasting material applications. However, identifying sustainable cascading pathways remains challenging due to inconsistent data, sector-specific models, and limited cross-sector integration.

The newly launched Swiss research project ACHIEVE develops an integrated framework for the valorisation of biomass in the technosphere, focusing on cascading use pathways, material substitution, and carbon storage. The project brings together leading Swiss research institutions and industry partners to establish a coherent national picture of biomass production, transformation, and use across sectors such as forestry, agriculture, industry, construction, and bioeconomy.

A first objective is to build a comprehensive overview of existing models and datasets used for biomass flow quantification in Switzerland and Europe. These include statistical inventories, material flow analyses (MFA), dynamic stock models, and life cycle-based approaches. By systematically mapping and comparing these models, ACHIEVE aims to identify synergies, inconsistencies, and data gaps, thereby supporting harmonisation across spatial, temporal, and sectoral boundaries. This model synthesis should provide the foundation for a consistent national assessment of biomass flows.

Building on earlier work, including the static MFA of woody biomass developed in the Joint Initiative SCENE, ACHIEVE extends the analysis to encompass major agricultural and industrial biomass resources within the technosphere. The integration of multiple modelling approaches will enable the transition from static representations to dynamic MFA, capturing regional changes in biomass stocks and flows.

By combining data integration, systems modelling, and stakeholder input, ACHIEVE seeks to identify promising cascading use pathways that maximise material valorisation and minimise emissions, while recognising that diverting biomass for material use may affect existing uses in other sectors and create trade-offs. ACHIEVE aims to assess these tensions transparently and engage the research community to refine methods, address gaps, and advance circular bioeconomy and net-zero strategies in Switzerland.

5.05.P-Tu425 Effects of Allocation on Nutrient Sharing Between Crops: A Comparative LCA of the Soybean–Cotton System in Brazil

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The allocation of nutrients from mineral fertilization remains a challenge in the Life Cycle Assessment (LCA) of agricultural systems. Agricultural studies commonly allocate all impacts and benefits of nitrogen (N),

phosphorus (P), and potassium (K) to the crop planted at the moment of application. This allocation approach is suitable for systems with a single annual crop, but it does not reflect contexts like Brazil, where two crops may be grown within the same agricultural year. Consequently, this practice is simplistic and overlooks soil-plant dynamics, characterized by the residual effect of phosphorus, the high mobility and leaching potential of potassium, and the low persistence of nitrogen in the system. As a result, this simplification may change LCA outcomes, influencing management decisions and conclusions regarding key environmental hotspots. In this context, the current study assesses the effect of various nutrient allocation approaches for N, P, and K in a soybean-cotton system with established soil fertility in Mato Grosso, Brazil. The analysis compares three scenarios: (i) full allocation of fertilization to soybean; (ii) equal distribution between soybean and cotton; and (iii) a proportional approach based on nutrient export and crop yield. In the latter scenario, the model fully allocates N to the receiving crop, allocates P according to nutrient export, and distributes K by crop in sandy soils or proportionally to export in clay soils, reflecting the differences in soil retention and mobility. The study models the inventory using the ICVCalc tool and calculates impacts in SimaPro software using the ecoinvent 3.11 database and the ReCiPe 2016 method. This research focuses on assessing the effects of eutrophication, acidification, climate change, and other categories directly linked to N, P, and K dynamics, as well as the differences in the results of each allocation approach. By demonstrating how allocation alternatives can change agricultural LCA results, the study highlights the need for allocation criteria that more consistently represent the biogeophysical processes of tropical systems, improving completeness and representativeness of fertilizer-related effects in LCA cases. The findings aim to support recommendations for allocating mineral fertilizers in life cycle assessments of Brazilian agriculture.

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5.05.P-Tu426 Towards a Transparent and Sustainable Atlantic Fisheries Sector: The SMART4SEA Approach

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The Atlantic fisheries sector, a cornerstone of the region's economy, culture, and food security, faces growing pressure to ensure environmental sustainability, traceability, and transparency across its value chains. Despite recent advances, the sector still struggles with fragmented data systems, limited interoperability, and the absence of integrated digital infrastructures and harmonized data protocols. These deficiencies restrict the flow of reliable information to authorities and consumers, hindering effective environmental management, enabling food fraud, and undermining public trust in seafood products. Addressing these shortcomings is therefore essential to enhance transparency and traceability, strengthen governance mechanisms, and ultimately secure the long-term resilience of Atlantic fisheries in a rapidly changing socio-environmental context.

In this context, the European project SMART4SEA proposes an innovative environmental traceability framework designed to enhance transparency and communication with consumers throughout the Atlantic fisheries sector. The project focuses on key priorities such as detecting fraud in fishery products, reducing the ecological footprint of extractive activities, mitigating marine litter, and improving resource efficiency. To achieve these goals, SMART4SEA integrates advanced digital technologies, circular-economy principles, and transnational cooperation among ten partners from four Atlantic-region countries, promoting knowledge exchange, the uptake of best practices, and ensuring the scalability and replicability of the developed solutions. As a preliminary outcome, a digital infrastructure is expected to be established in the coming months to integrate real-time data from the fisheries under analysis.

The expected outcomes of SMART4SEA will reinforce data-driven traceability and environmental responsibility in value chains that are strategic for both food security and regional economic growth. The project will deliver innovative tools for producers and processors, while empowering consumers with clear, trustworthy

environmental information supporting more responsible choices and contributing to a resilient, sustainable blue economy.

5.05.P-Tu427 From Waste to Fuel: Life Cycle Insights into Biogas from Winery By-products

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Wineries generate large volumes of organic residues whose management represents both an environmental challenge and a resource recovery opportunity. Anaerobic digestion (AD) offers a circular solution for these streams; however, little is known about how different winery residues compare environmentally under harmonized assessment conditions. This study performs a comparative gate-to-gate Life Cycle Assessment (LCA) of two AD pathways: (A) biogas production from 1 m³ of winery wastewater, and (B) biogas production from 1 tonne of grape pomace. Inventory data were compiled from recent experimental and literature sources and modelled in OpenLCA using ecoinvent 3.10. Environmental impacts were evaluated using the ReCiPe Midpoint (H) method.

To enable comparability, results were expressed using both substrate-based functional units and an energy-based functional unit (MJ of useful energy, derived from methane yield and lower heating value, or LHV). Preliminary results indicate that grape pomace can produce higher biogas yields; however, the upstream energy need, due to the digestion time and pre-treatment requirements, result in higher impacts per unit of substrate. Conversely, winery wastewater offers a lower methane potential but contributes to net environmental benefits through reduced organic load, such as eutrophication and aquatic toxicity. Sensitivity analyses demonstrate that methane content, digester heating demand and biogas utilisation route strongly influence the ranking of scenarios.

This work provides a comparative LCA for bioenergy production using solid and liquid winery residues. The findings support evidence-based decisions for sustainable waste-to-energy strategies in the wine sector and emphasise the importance of functional unit selection in comparative LCA studies.

Keywords: anaerobic digestion; biogas; grape pomace; winery wastewater; LCA; circular bioeconomy

5.05.P-Tu428 Enhancing Environmental Accounting: An Exploratory Approach to Soil Stocks in Agricultural Life Cycle Assessment

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Agricultural Life Cycle Assessment (LCA) is not a closed system. Modeling agricultural processes requires dealing with edaphoclimatic variability. A key variability aspect is the presence of soil stocks (such as phosphorus, heavy metals and water), which form part of the biophysical context in which agricultural activities occur. These stocks influence how processes should be modeled and interpreted within LCA. Yet current LCA databases and modelling approaches often treat these stocks implicitly or as background conditions. As a result, agricultural datasets become highly dependent on the initial context in which they were generated. Based on this premise, this work aims to examine LCA database models to understand how soil stocks are currently modeled. Consider phosphorus (P), for example, a critical contributor to freshwater eutrophication. Many tropical soils have more adsorption of P, which prevent their leaching. Soils with high levels of available P require less or even no phosphate fertilization, respecting the crop's needs, while soils poor in available P demand higher doses of fertilizer. However, applying little or no phosphate fertilizer now does not necessarily mean a more sustainable practice, because past over-fertilization has already raised soil P levels to very high values. Thus, agriculture should work with target values for soil nutrient stocks, since a good practice requires preventing both shortages and accumulation. Moreover, erosion-driven P losses depend on the current stock level (whether past or newly applied), meaning emissions may appear higher simply due to geographic or pedological conditions rather than actual fertilization practices. As stocks approach environmental thresholds, high level P may also

trigger higher P exports. Similar complexities arise for other soil stocks such as water and heavy metals. Thus, without explicitly accounting for soil stocks, LCA results may ambiguously represent environmental performance, merging management impacts with geographic or pedological characteristics. This also weakens comparability across datasets, since differences in stock dynamics are not adequately reflected in flow values. The findings obtained from this review can support the development of modelling approaches that reduce the context-dependency of agricultural LCA datasets and improve comparability between datasets, yielding more robust environmental assessments while remaining consistent with core LCA principles.

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5.05.P-Tu429 Exploring Outliers in Large-Scale Life Cycle Assessments of Experimental Farms

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Experimental farms are able to test a large range of management practices. Even if trials are not conducted with the aim of improving sustainability, focusing instead on yield improvement or climate adaptation, for example, they can still reveal potential methods of reducing environmental impacts. Life cycle assessment (LCA) is a key tool for assessing the sustainability of farming practices. The covariance between the resulting environmental impacts and cultivation variables, e.g., the quantity of fertiliser applied, can reveal potential mitigation options. However, focusing on covariance alone means that outliers may not be fully considered (e.g., if their weight in the correlation is low) or may be excluded from some analyses. This research focuses on these outliers, to see what lessons can be learned from them and how they can be included in the search for impact-reducing practices. To show how this can be done, we are using the Sustainable Corn CAP (Cropping Systems Coordinated Agricultural Project) Research Data. The data was collected between 2011 and 2015, covering eight states in the US, and containing over 3,000 production cycles of maize, soybean, and wheat. Treatments used in the experimental trials include varying practices such as cover crops, fertiliser applications, crop rotations, drainage systems, and tillage regimes. The data also contains measured soil information (e.g., carbon and nitrogen contents), allowing for a more accurate calculation of a cycle's soil-related emissions, such as carbon dioxide emissions from soil carbon stock changes. This dataset has been uploaded onto HESTIA to conduct the LCAs, a platform that makes food production and impact data available in a standardised format. HESTIA contains over 1000 models which calculate emissions and resource uses, producing multi-indicator impact assessments for each cycle in the dataset. We analyse the contributions of emissions to each indicator for the outlier farms; assess what drives these impacts; and pinpoint the reasons behind each outlier. The multi-indicator assessment also allows us to analyse trade-offs between indicators and understand why they exist. Our work ensures that we can capture the benefits of novel practices and alternative technologies when designing farm advice by focusing on outliers and not simply excluding them.

5.06.P Energy Systems in Transition Present New Challenges for LCA Practitioners and Researchers

5.06.P-Mo343 Environmental Evaluation of Solid-State Battery Manufacturing Using a Cradle-to-Gate LCA Approach

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The development of next-generation battery technologies is driven by the need for higher energy density, improved safety, and more sustainable production routes. Solid-state batteries that combine lithium metal anodes with high-nickel cathodes have emerged as promising candidates, yet the environmental impacts of their manufacturing processes are not fully assessed. This study addresses this gap by evaluating the environmental performance of an experimental solid-state battery based on an NMC811 cathode and solid polymer electrolyte, and comparing it with a state-of-the-art NMC622 lithium-ion battery using a liquid electrolyte.

In the solid-state configuration, the graphitic anode is replaced with lithium metal to increase energy density. Since lithium metal is unstable in liquid electrolytes due to dendrite formation, solid polymer electrolytes are selected to improve interfacial stability and enable safe cycling. The primary objective of the study is to quantify

material and energy demands associated with producing key solid-state battery components and to identify the dominant contributors to environmental burden.

The methodology combines laboratory-scale fabrication of dry-processed composite cathodes, thin lithium metal anodes, and polymer electrolytes with a cradle-to-gate life cycle assessment. Standard LCA frameworks are used to evaluate material flows, energy consumption, and environmental impacts across all relevant processing steps. Questionnaires have been distributed for inventory buildup to obtain process-level data for solid electrolyte synthesis, polymer formulation, and lithium metal handling in order to ensure representative inventory inputs.

Preliminary insights already indicate environmental hotspots associated with raw materials such as cobalt, solid electrolyte production, and energy-intensive lithium metal processing, including coating steps. Detailed quantification of these impacts will be carried out in the subsequent stages. Identifying these hotspots will support strategies to reduce the environmental footprint of solid-state batteries.

Understanding the implications of transitioning from conventional lithium-ion to solid-state architectures is essential for guiding sustainable battery development. The outcomes of this work will inform process optimization and design decisions that advance solid-state battery technologies while maintaining environmental responsibility.

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5.06.P-Mo344 From Charging to Sustainability: Assessing Use-Phase in Battery Life Cycle Assessment

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Life Cycle Assessment, LCA, is a robust methodology for evaluating the environmental impacts of products across all life stages, from raw material extraction to end-of-life. In the context of battery systems, the use phase is particularly significant, as it directly influences the overall energy demand and associated emissions.

Accurately representing this phase requires a detailed understanding of battery performance throughout its lifetime, including charging energy requirements, which are affected by degradation and efficiency losses.

This work proposes a structured approach to quantify the energy input during the operational life of a battery module, enabling a realistic estimation of its environmental footprint. Key parameters, such as initial and aged capacity (Ah), average voltage (V), charging efficiency at the beginning and end of life (%), and number of cycles, are obtained through experimental testing. These parameters allow the calculation of energy per cycle and total energy delivered over the battery's lifetime, considering both initial and aged conditions.

The methodology involves computing the initial and aged energy per cycle, multiplying by the respective number of cycles, and adjusting for charging efficiency to determine total energy delivered. Summing these values provides the lifetime energy input, which, when combined with the regional electricity mix, enables the assessment of environmental impacts such as CO₂ emissions per kWh.

By integrating this use-phase evaluation into the broader LCA framework, the approach ensures a comprehensive representation of operational impacts, supporting informed decisions in battery design and sustainability strategies.

This work will be further developed and will therefore contribute to a complete life cycle analysis of battery systems and their role in reducing environmental burdens in electric mobility.

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5.06.P-Mo345 Life Cycle Assessment of Hybrid Energy Storage Systems for Maritime Electrification: Environmental Trade-offs and Benefits

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Maritime transport is a major and growing source of greenhouse gas emissions, projected to increase by up to 130% over 2008 levels by 2050. Electrification is a key strategy for reducing emissions, enabling high-efficiency propulsion systems powered by renewable energy. However, the adoption of Battery Energy Storage Systems (BESS) in maritime applications is limited by mission profiles with high dynamic loads and long durations, requiring significant oversizing of storage systems, which increases mass, volume, and costs. Hybrid Storage Systems (HSS), combining batteries with supercapacitors (SC) or Superconducting Magnetic Energy Storage (SMES), offer an effective solution to manage power transients and reduce thermal and electrical stress on batteries, improving efficiency, lifetime, and reliability. This approach was investigated in the European project Vessel Advanced Clustered and Coordinated Energy Storage Systems (V-ACCESS), which included a dedicated task applying the Life Cycle Assessment (LCA) methodology (ISO 14040/14044) to estimate the potential environmental benefits of hybrid systems compared to battery-only configurations.

Three representative marine applications were analysed: a fully electric ferry, an Offshore Supply Vessel with Active Heave Compensation and a trawler. For each, several configurations were assessed considering different size of the BESS, SC and/or SMES. The functional unit for the LCA was one hour of mission over a 30-year of vessels lifetime. The scope covered storage systems, including replacements, energy losses during charge/discharge (considering efficiency degradation over 30-years), and cooling-related losses. System boundaries followed a cradle-to-grave approach, from raw material extraction to end-of-life treatment. Environmental impacts were assessed using the EF 3.1 method. Life cycle inventory was built based on both primary/simulated data and secondary data. Specifically, foreground processes were modelled based on data provided by V-ACCESS industrial partners; background processes were modelled based on Ecoinvent 3.9.1 database.

The LCA quantified life cycle impacts of hybrid storage adoption and potential benefits over battery-only systems. Analysis of different configurations identified the most suitable solution for each application. A key contribution to eco-design came from the analysis of individual process contributions to overall impacts.

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5.06.P-Mo346 How to Build State-of-the-Art Life Cycle Inventory Models for Renewable Energy Systems

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The growing deployment of renewable energy technologies such as wind and solar power as well as public criticism about their potential environmental impacts; has increased the need for reliable tools to evaluate their environmental performance. Life Cycle Assessment (LCA) is widely used, but its accuracy depends on access to detailed technical data that feed the Life Cycle Inventory (LCI). The potential environmental impacts of renewables vary depending on the system manufacturer and model. However, exhaustive technical data allowing to build full LCI of different renewables systems is often confidential or unavailable, making representative LCAs difficult to compute.

Manufacturer and model-specific technical data can be available in databases, scientific literature or reports of companies, making it possible to build some mathematical models to predict missing LCI data. Several models have been proposed in the literature, although useful, these models are not always statistically justified and do not consistently quantify the uncertainty in their predictions, raising questions about their reliability.

This work presents a clear and structured methodology for building state of the art models for energy systems LCI prediction when technical data are scarce. The approach includes screening LCA, to identify key contributors, correlation analysis to select the most relevant parameters, regression models to estimate key dimensions and masses, and explicit integration of uncertainties. Uncertainties from both model parameters and prediction errors are then propagated through the LCI using Latin Hypercube Sampling, providing confidence intervals for all outputs.

The methodology is applied to a cradle-to-grave LCA of a fictitious 6 MW onshore wind turbine installed in Île-de-France, Franch region, assuming only its nominal power is known. After uncertainty propagation, the climate change impact is estimated at a median of 14.9 g CO₂-eq/kWh, with a 90% confidence interval of [9.8; 22.2].

This demonstrates how limited technical information can significantly increase uncertainty in LCA results.

Overall, this framework improves the robustness and transparency of LCAs for renewable energy systems under data scarcity. It can support large-scale impact assessments and early-stage evaluations of future installations.

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5.06.P-Mo347 A Life Cycle Assessment of Sustainable Aviation Fuel (SAF) Produced from Indonesian Coconuts

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Following the Paris Agreement and the commitment of many countries to achieve carbon neutrality by 2050, it has become clear that emission-reduction technologies alone are not sufficient to meet long-term climate goals. A deeper transformation of the energy system is required, and the aviation sector faces particularly complex challenges.

Sustainable aviation fuel (SAF) has emerged as a promising option for reducing aviation emissions. However, its environmental performance varies widely depending on the feedstock, land-use conditions, and production requirements. In this case, Indonesia with its abundant coconut resources, has recently attracted attention as a potential supplier of coconut-oil-based SAF via the Hydroprocessed Esters and Fatty Acids (HEFA) pathway. Yet, comprehensive and transparent life cycle assessments of coconut-derived SAF remain limited.

This study evaluates the emissions of SAF produced from Indonesian coconut oil and examines how land-use assumptions, agricultural practices, and HEFA processing conditions influence overall results. The assessment integrates primary data collected from Indonesian coconut-oil producers with process data obtained from research institutions in Japan, allowing for a more realistic representation of the production system. Multiple land-use change (LUC) scenarios are modeled to reflect plausible development pathways.

The analysis adopts a cradle-to-gate system boundary, covering coconut cultivation, crude coconut-oil extraction, hydrogen and electricity use in the HEFA process, and allocation of co-products. Sensitivity analyses further investigate the effects of hydrogen sourcing, electricity mixes, and LUC assumptions on total emissions. Preliminary results indicate that coconut-based SAF can reduce emissions substantially compared with conventional jet fuel in several scenarios. However, the magnitude of these reductions depends strongly on land-use dynamics and the energy mix used during HEFA processing.

Overall, this study highlights the importance of integrating region-specific primary data and robust land-use modeling when assessing emerging bio-based fuels. As one of the first systematic evaluations of Indonesian coconut-derived SAF, the findings offer insights that may support sustainable feedstock selection, aviation decarbonization strategies, and broader international energy-transition efforts.

Disclaimer/Disclosure:

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5.06.P-Mo348 Clean Fuel, Dirty Build-Out? Quantifying Infrastructure Erosion of Hydrogen's Climate Benefit in Short-Haul Aviation

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Hydrogen continues to attract attention as a potential low carbon aviation fuel, yet the conditions under which it truly delivers climate benefit are often assumed rather than tested. The fundamental barrier is volumetric storage. Conventional airframes place fuel within the wings, achieving large capacity with minimal intrusion into the cabin or hold. Liquid hydrogen cannot be stored this way due to its cryogenic nature and the surface area constraints required to limit boil off. Most proposed airframes therefore relocate fuel into cylindrical or toroidal tanks within the fuselage. This architecture imposes a direct penalty on either payload or range. As the energy requirement increases the tank volume expands, progressively displacing revenue generating space. Hydrogen aircraft are therefore likely to be viable only for short range missions where fuel demand is modest and fuselage intrusion remains tolerable.

That physical feasibility interacts problematically with infrastructure. Long haul flights are concentrated at major hubs. In contrast, short haul operations form dense, decentralised networks spanning many regional airports. If hydrogen is adopted for these segments the supporting fuel infrastructure must proliferate across

orders of magnitude more locations than would be required for long-haul hub centred deployment. The environmental cost of producing, distributing and storing hydrogen at this scale is poorly understood and may offset aircraft level emissions savings.

This work applies a cradle to grave life cycle assessment with system expansion to estimate the net climate outcome of hydrogen based short haul aviation in Europe. The model includes centralised production and mixed pipeline and tanker distribution, followed by airport storage, handling, refuelling and use phase substitution of fossil kerosene. Results are reported as net GWP for one year of European short haul flights and as a seat kilometre intensity metric. The study interrogates whether infrastructure demand materially erodes climate benefit and determines the conditions under which hydrogen powered aviation remains environmentally defensible at continental scale.

5.06.P-Mo349 Assessing the Environmental Impacts of a 10 kWel Coupled System Based on Metal Hydride Storage

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The need to reduce greenhouse gas emissions is driving the development of renewable electricity systems and increasing the demand for effective large-scale energy storage solutions. Hydrogen is recognized as a key energy carrier for enabling long-duration energy storage, but challenges remain for large-scale solid-state hydrogen storage technologies. Helmholtz-Zentrum Hereon addresses these challenges via the Digi-HyPro project, developing integrated medium- and large-scale hydrogen systems based on commercially available AB₂-type hydride alloys. This study presents a cradle-to-grave attributional life cycle assessment (LCA) of an integrated hydrogen production, storage, and use system currently under construction. The system includes a 9.6-kW net-power anion-exchange membrane electrolyzer, a 1-kg H₂ metal-hydride storage module, and a 5-kW net-power proton-exchange membrane fuel cell. While most existing LCAs of hydrogen storage technologies focus primarily on CO₂-equivalent emissions and compressed-gas systems, this work provides a more comprehensive evaluation of a solid-state storage approach across a broader set of environmental impact categories.

Life cycle modeling is performed in Umberto using ecoinvent 3.11. The system boundary covers the materials and manufacturing of the metal-hydride tank, electrolyzer, and fuel-cell stack; balance-of-plant components; operational electricity demand; and thermal energy requirements for hydrogen absorption and desorption. Environmental impacts are assessed using ReCiPe 2016, examining global warming potential, mineral and metal resource use, and selected human-health and ecotoxicity indicators. To contextualize metal-hydride performance, the system is compared with a modeled 300-bar pressure tank as a conventional compressed-hydrogen baseline. Scenario analyses are conducted for a range of electricity supply conditions, from the current grid mix to highly renewable and fully renewable electricity scenarios. The study aims to identify the most relevant contributors to environmental impacts across the assessed categories and to clarify trade-offs between system efficiency, material requirements, and environmental performance. By assessing solid-state hydrogen storage within an integrated system and considering impacts beyond climate change, this work fills an important gap in the LCA literature and supports informed decision-making for future hydrogen storage technologies aligned with sustainability goals.

5.06.P-Mo350 Climate-optimal deployment of green hydrogen

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Green hydrogen is projected to play a key role in achieving net-zero emissions, with applications across various sectors. While hydrogen applications have been assessed on costs, competitiveness and feasibility, it is unclear which applications are most favourable for the climate. Here, we use prospective life cycle assessment to compare the greenhouse gas emissions of green hydrogen use in various applications with: i) their fossil counterparts, and ii) low-emission alternatives for these applications, which are other mitigation technologies that provide the same service. Specifically, we look at methanol, ammonia, steel, aviation fuel, passenger cars, long-term grid balancing, and domestic and industrial heat production for the year 2030. We demonstrate that

green hydrogen production, transport and application leads to emissions savings compared to their fossil counterparts, but emissions are similar or higher than those of the low-emission alternatives. Only with very low hydrogen production emissions and without transport do the green hydrogen-based applications result in net emissions savings compared to the low-emission alternatives for: ammonia, steel, long-term grid balancing, and industrial and domestic heat. We conclude that for green hydrogen to fulfil its anticipated role in the net-zero transition, emission reductions are needed across the supply chain, as well as a prioritisation of hydrogen use in different applications that accounts for and optimises climate benefits.

5.06.P-Mo351 Quantifying Life-Cycle Stage Burden-Shifting Intensity in Global Warming Potential Induced by Post-Combustion Carbon Capture and Storage for Decarbonizing Energy-Intensive Industries

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Energy-intensive industries (EIIs), together with the emissions arising from their energy supply, account for around 60% of global greenhouse gas releases, making their decarbonisation critically important. Post-combustion carbon capture and storage (PCCCS) is widely considered a key mitigation option due to its broad applicability, modest retrofit requirements, and diverse technological choices. However, from a life-cycle perspective, PCCCS is itself energy- and material-intensive, potentially generating additional CO₂-equivalent emissions that offset part of its CO₂ mitigation benefits.

To elucidate the systemic challenge of hidden yet critical life-cycle burden shifting induced by PCCCS, this study introduces the parasitic Global Warming Potential (GWP) penalty rate, a metric designed to quantify the intensity of life-cycle stage burden shifting in GWP induced by PCCCS—that is, the extent to which climate burdens move from the capture stage to other life-cycle stages. Using 80 meta-data points from 43 studies published between 2015 and 2025, we systematically quantified this indicator.

For PCCCS in EIIs, the parasitic GWP penalty rate exhibits an interquartile range (IQR) of 9%–27%, with the mean value at 19%; Specifically, adsorption (IQR 21%–37%, mean 31%) > absorption (8%–26%, 18%) > calcium looping (8%–27%, 15%) > membrane (8%–20%, 15%). These findings show that parasitic burdens represent a non-negligible component of the overall GWP results of PCCCS, highlighting the need for their explicit consideration in life-cycle assessments and for further work to identify mitigation priorities.

5.06.P-Mo352 Time-explicit Life Cycle Optimization for Transition Pathways: An Open-Source Python Implementation

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Achieving environmental sustainability requires transforming existing anthropogenic systems over time. Transition pathway optimization supports this transformation by identifying the optimal scale and timing of technological choices based on defined objectives such as minimizing environmental impacts. To quantify environmental impacts of technologies, these optimization problems are often linked with Life Cycle Assessment (LCA) in so-called Life Cycle Optimization (LCO). However, in conventional LCA, life cycle stages of technologies, and their elementary flows, intermediate flows and environmental impacts, are aggregated over time. This overlooks the temporal distribution of life cycle stages, the temporal evolution of processes within life cycle stages and the temporal accumulation of emissions environmental impacts over time.

To overcome these limitations, we combine transition pathway optimization with time-explicit LCA, which introduces time as an additional dimension in life cycle modeling. To this end, we expand the conventional two-dimensional LCA matrices into three-dimensional tensors. Thereby, we enable the consideration of temporally dynamic scaling of processes, the processes' elementary and intermediate flows, as well as characterization factors. With this new tensor structure, the temporal distribution of life cycle stages, the temporal evolution of processes within life cycle stages and the temporal accumulation of environmental impacts can be considered. By further implementing the new tensor structure in LCO, we enable the determination of the optimal scale and timing of technology deployment to achieve minimal cumulative environmental impact.

We present an open-source implementation of this method in the Python package Optimesx, which extends the Brightway LCA framework, integrating Pyomo for mathematical optimization. Optimesx provides a unified structure for time-explicit life cycle modeling and LCA-based transition pathway optimization, enabling users to define optimization problems that consider time-resolved life cycle inventories and time-resolved life cycle impact assessment. We demonstrate the functionalities of Optimesx through a transition pathway optimization of

an industrial energy system, showcasing how time-explicit modeling enables more representative, forward-looking sustainability assessments.

5.06.P-Mo353 Digitalising Environmental Transparency for Renewable Energy: Sensitivity and Hotspot Analysis of Blockchain-Enabled Traceability of Solar Photovoltaic Panels Life Cycle

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Europe's pathway toward climate neutrality by 2050 increasingly relies on robust, high-quality environmental information capable of supporting sustainable product design, transparent supply chains, and verifiable environmental claims. Upcoming regulatory frameworks, including the Ecodesign for Sustainable Products Regulation, the Corporate Sustainability Reporting Directive with disclosure obligations requiring the introduction of Digital Product Passports and even sector-specific traceability requirements such as the EU Battery Passport, pave the way for a transition toward digitally enabled lifecycle data systems. The frameworks aim to improve the reliability, auditability, and granularity of environmental information while reducing risks of greenwashing and strengthening trust across value chain actors. As Europe moves toward digital environmental data ecosystems, there is growing interest in digital traceability architectures capable of capturing product-specific lifecycle information. The present study adopts the Life Cycle Assessment methodology in line with ISO 14040 to investigate how the environmental performance of a blockchain-enabled digital traceability system is influenced by variations in system parameters across its lifecycle. The case of Solar Photovoltaic Panels Life Cycle is selected as object of the study. By combining sensitivity analysis with threshold identification, the study highlights the trade-offs between increased data transparency and the material/energy intensity of the digital infrastructure required to achieve it. Findings provide insights into the design of environmentally responsible digital transparency systems and inform policy discussions on how to ensure that the digitalization needed to document Europe's progress toward climate neutrality itself does not create disproportionate environmental burdens.

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5.06.P-Mo354 Circularity Assessment of the Electricity Sector: Development of an LCA-Based Metric

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In the context of the European Union energy transition, circular economy models play a central role in strategies for competitiveness and decarbonisation. Circular business models, such as extending product lifespans and resource recovery, help mitigate risks related to energy security and the supply of critical minerals essential for green technologies. Effective action requires metrics to assess the current state and monitor progress towards a more circular energy sector.

Within this framework, the research focused on developing an improved version of a circularity metric for the electricity sector at macro-scale. Building on a previous tool, it was redesigned to comply with RACER criteria (Relevance, Acceptance, Credibility, Ease, Robustness) defined by the Bellagio Charter, the European reference for circularity indicators. Particular attention was given to ensuring that all indicators are based on reliable and verifiable data, mainly official statistics, and to improving data availability and temporal consistency. These enhancements guarantee continuity and annual accessibility of statistical series, essential for multi-year monitoring.

The metric is structured into three areas: enabling factors (policies, innovation), sector structure and operations (technology configuration, material and energy flows), and environmental effects, assessed through the Life Cycle Assessment methodology (ISO 14040/14044). The circularity score classifies electricity systems as "beginner", "started", "concerned" or "circular". Applied to the Italian electricity system for 2023, with reference to 1 kWh of gross domestic consumption (excluding transmission), the metric shows a "consolidated" status in the circular transition. Annual monitoring, retroactive to 2021, highlights stability, confirming sector maturity.

The metric, among the few tools available in the literature for assessing electricity system circularity, can be updated annually using official and scientific data, enabling rapid assessment of the system's evolution and supporting targeted actions to enhance circularity. It can also be integrated as an additional parameter within broader sustainability assessment frameworks. Future developments include extending system boundaries to

cover transmission and evaluating applicability to other European Union Countries. However, the need for a continuously updated digital system is emphasised, capable of quickly collecting data across all dimensions of circularity.

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5.07 How Low Should You Go? Recent Developments in Absolute Environmental Sustainability Assessment

5.07.T-01 Absolute Environmental Sustainability Assessment of Global Economy via Environmentally Extended Input Output Analysis

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Industrialisation and rising consumption have pushed seven of nine Planetary Boundaries (PBs) beyond safe ecological limits. Absolute Environmental Sustainability Assessment (AESA) is a novel approach to evaluate whether systems operate within these limits. However, the evolution of overshoot across different economies over time, and the role of global supply chains in driving this overshoot, remain poorly understood. Here, we perform an AESA across 37 high-income and 12 middle/low-income economies over 1995-2020 using Exiobase v3.9.6 and a newly developed PBs-based impact assessment method (PB-IA), evaluating 9 Earth-system indicators across 7 PBs under 4 equity-based allocation approaches.

Results show that environmental decoupling from economic growth is uneven and insufficient across economies and PBs, with no economy achieving absolute environmental sustainability. Decoupling is observed mainly in high-income economies—particularly for climate change, land-use, and biodiversity-related PBs, but these economies account for only ~20%-35% of global impacts. In contrast, middle/low-income economies—responsible for ~65-80% of global impacts, remain strongly coupled to major environmental pressures. Global supply chains increasingly redistribute environmental pressures through trade, but domestically produced impacts remain dominant, accounting for ~68%-78% of impacts across PBs. Over time, impact reductions in high-income economies are largely driven by foreign impact intensities reduction (efficiency and technology improvements abroad). Conversely, rising impacts in middle/low-income economies are mainly driven by growing domestic consumption.

While global supply chains have redistributed environmental impacts across economies and enabled relative decoupling in high-income economies, they have not delivered absolute environmental sustainability. Demand-side shifts in high-income economies with technology, finance, and capacity-building for middle/low-income economies is essential for pushing the world towards absolute environmental sustainability while ensuring equitable development.

5.07.T-02 Evaluating Global Hydrogen Supply Pathways under Resource Limits through Cost and Planetary Boundaries Minimisation

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Hydrogen is seen as an energy carrier for the transition to a low-carbon future. While today's hydrogen production is predominantly fossil-based and centralised, future production and demand are likely to decouple. Regions with abundant renewable energy can supply low-cost green hydrogen, while others face high demand but limited local potential. This mismatch introduces significant challenges across the supply chain, including resource constraints, transport logistics, and environmental thresholds defined by the Planetary Boundaries (PBs) framework.

To address these gaps, we developed a scenario-based, multi-objective Mixed-Integer Linear Programming (MILP) model to optimise global hydrogen supply chains under projected 2050 conditions. The model includes 46 production technologies, ranging from conventional methods to emerging approaches using waste and biomass as feedstock. We included 25 transport options combining different hydrogen carriers and transportation means. Hydrogen trade is allowed across 12 world regions, with region-specific resource limits. Furthermore, our analysis spans hydrogen demand scenarios from 138 to 614 Mt/year and considers resource

allocations from 10 to 100%.

The system is optimised under two objectives: cost minimisation and minimisation of the Planetary Boundary Footprint (PBF), an aggregated indicator reflecting boundary transgressions across ten Earth-system processes. To distinguish between solutions achieving the same PBF, we fix its value and maximise the Planetary Boundary Budget (PBB), a novel metric that reflects the remaining safe operating space across all boundaries. In both cases, environmental impacts were calculated using a prospective life cycle assessment aligned with the Planetary Boundaries-Life Cycle Impact Assessment framework.

Results show that under a 10% resource allocation, only 326 Mt H₂ can be supplied. This quantity remains below IEA and IRENA estimates. Cost-optimal solutions tend to rely mostly on fossil feedstocks and some biomass, increasing pressure on PBs such as Nitrogen flow and CO₂-based control variables. In contrast, PBF-optimised pathways favour nuclear-powered water electrolysis, reducing key environmental impacts, yet at higher costs.

Despite all the options considered, a fully green hydrogen system by 2050 is infeasible due to resource constraints, which highlights the need for region-specific strategies that balance costs, technologies, and Earth-system limits.

5.07.T-03 Defining Sustainability Targets for Mobility and Batteries

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Delivering mobility services within the safe operating space (SOS) will be complex as passenger mobility demand is expected to triple to 183 trillion passenger-kilometres in 2050. Therefore, assessing safety limits during the design phase of mobility services is imperative. We propose a methodology to downscale the SOS to mobility sub-sectors and ultimately product component, demonstrated with electric vehicle batteries. We compute targets for 2030, 2035 and 2050, and perform an uncertainty analysis based on a multiplicity of allocation factors and study parameters, including location, driving intensity, battery capacity and lifetime. We conduct our case study in Germany and Canada to assess how travel intensity and reduced car dependency affect target distributions. We find similar targets for both countries due to comparable mobility requirements and anticipated decarbonization of grid mix. Opting for a mini versus a mid-size car permits ~40-50% higher mean targets per kWh. For a 72-kWh battery pack, mean targets (and 5th to 95th percentiles) for the year 2030 stand at 10 (1-27) kg CO₂-eq/kWh and 1 (0.2-2) m³ water/kWh. Unless the yearly travel distance in both Germany and Canada is significantly decreased, increasing the share of public and active mobility is necessary in order to meet calculated sustainable mobility targets. Our target distributions facilitate their operationalization by moving beyond a yes/no assessment of SOS compliance, providing valuable insights for policymaking.

5.07.T-04 Remote-Sensing-Supported Planetary-Boundary-Based Absolute Environmental Sustainability Assessment. A case study for Iron Ore Regions

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Absolute Environmental Sustainability Assessment (AESA) asks whether activities are compatible with a fair share of the Earth's carrying capacity. While the planetary boundaries (PB) framework provides a science-based reference, its operationalisation at regional and sector levels and its integration with life cycle assessment (LCA) remain limited, particularly where data are scarce.

We develop a PB (planetary boundaries)-based AESA framework for iron ore regions that directly evaluates PB control variables and systematically combines remote sensing and complementary datasets with conventional LCA data. First, we implement a prototype assessment for Kiruna (Sweden), using globally consistent gridded data to characterise regional pressures on selected PBs and to downscale the global safe operating space. This first case, now completed, focuses on regional environmental status rather than a full product LCA and serves to identify methodological gaps, including sensitivity to spatial boundaries and the interpretation of highly impacted urban areas (focus on remote sensing and global available datasets). Results for Kiruna indicate local transgressions for several PB indicators and reveal spatial hotspots, but also show that concentrated urban/industrial impacts may coincide with comparatively intact surrounding ecosystems. This questions simple per-area or per-capita downscaling and highlights risks of selective boundary setting if regional units are not defined transparently.

In a second step, we extend the framework in a full AESA of cradle-to-gate iron ore production in Eisenerz

(Styria, Austria). Here, site-specific life cycle inventory data are combined with spatially explicit PB indicators, an improved allocation scheme, and additional remote sensing and statistical datasets where LCA databases lack coverage. This enables a more comprehensive assessment of PBs and links regional conditions to production-related pressures.

Together, the two case studies provide a coherent storyline from framework setup and first testing to a more comprehensive AESA implementation. We conclude that PB-based AESA supported by remote sensing and extended data sources is feasible and yields useful insights, while requiring thoughtful interpretation of spatial heterogeneity and functional land use. The proposed approach reduces dependence on indirect LCIA-to-PB conversions and offers a template for spatially explicit absolute environmental sustainability assessments.

5.07.P How Low Should You Go? Recent Developments in Absolute Environmental Sustainability Assessment

5.07.P-Th204 A framework for combining prospective life cycle assessment and Absolute Environmental Sustainability Assessment (pAESA) through integrated assessment models

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Emerging technologies are essential to reduce human pressures on Earth's ecosystems. A key challenge is ensuring these technologies operate within planetary carrying capacities. Prospective life cycle assessment (pLCA) estimates future eco-efficiency under socioeconomic transition pathways but lacks explicit context relative to planetary limits. Absolute environmental sustainability assessment (AESA) evaluates impacts within these limits to make absolute sustainability claims but has mainly focused on current technologies with limited use of future scenarios. This study bridges that gap by integrating pLCA and AESA using harmonized scenarios from integrated assessment models (IAMs) to evaluate whether novel technologies—e.g. green hydrogen production—can operate within allocated carrying capacities in the future.

Future-oriented scenarios are central to pLCA. Temporal alignment of foreground and background systems through IAM integration using tools like premise is standard practice. This provides a strong foundation to unify pLCA and AESA by consistently applying IAM outputs across all AESA steps, drawing on the AR6 scenario database.

A key feature is dynamic, time-explicit implementation that aligns emission trajectories with prospective pathways. The dynamic functional unit drives a matching life cycle inventory. IAM data defines the climate change carrying capacity using the SSP1-1.9 CO₂ gross emission trajectory as baseline; other carrying capacities are treated as static.

Allocations of carrying capacities are based on regional GDP, population, energy demand, and emissions from AR6 data. This supports various allocation principles i.e. grandfathering, equal per capita, historical responsibility, capability to reduce, economic value, needs fulfillment, cost optimization and sufficiency lifestyles. Results are interpreted at multiple time points to capture temporal evolution.

The framework is idealized and reveals limitations due to the current state of the research. IAMs' climate focus may bias AESA; mismatches between carrying capacity scenarios and those used in pLCA and sharing principles need resolution. IAMs' neoclassical economic assumptions challenge equitable sharing. Static impact factors limit capturing ecosystem dynamics; inter-boundary interactions lack comprehensive integration for holistic sustainability. Case studies like green hydrogen production are needed to test and refine this framework's real-world applicability and effectiveness.

5.07.P-Th205 Additivity of Impacts Matters: Integrating Responsibility Principles in AESA of Economic Sectors

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Absolute Environmental Sustainability Assessment (AESA) aims to operationalise the planetary boundaries framework at decision-relevant scales, such as economic sectors. The downscaling step in AESA requires strict additivity: All allocated SOS (aSOS) must equal the global SOS. Just as the downscaling step has to ensure aSOS additivity, the environmental impact assessment must preserve the additivity of impacts to comply with the defined aSOS. However, from a life cycle perspective, this additivity is not guaranteed, particularly if the

study focuses on an intermediate sector in a value chain.

This work proposes a consistent methodology to ensure additivity in both the downscaling and environmental assessment steps of AESA for economic sectors. It relies on three complementary accounting approaches based on different principles of responsibility, i.e. production-based (PBA), consumption-based (CBA), and income-based (IBA).

PBA assigns responsibility for direct impacts of a sector. This approach is geographically intuitive, but does not account for emissions embodied in international trade.

CBA assigns responsibility to final consumers by attributing upstream embodied impacts. This approach is widely used in AESA and captures global supply-chain interdependencies, but it limits the ability to assess the responsibility of intermediate sectors.

IBA assigns responsibility to primary suppliers by attributing downstream induced impacts. This allows to study the environmental responsibility of sectors not directly linked to final demand. This approach is therefore complementary to CBA.

Sharing principles adopted follow the responsibility principle associated with each approach: sales turnover for PBA, final consumption expenditure for CBA, and value added for IBA. The methodology is applied to the country-level economic sectors of EXIOBASE, a multi-regional environmentally extended input-output table, for the climate change as a proof of concept.

Results highlight that IBA can be incorporated into AESA alongside PBA and CBA. This allows for a more complete assessment that does not focus only on final consumers.

The proposed methodology contributes to improving the consistence and completeness of AESA applied to economic sectors. By ensuring additivity of two main steps of AESA, it advocates for the standardisation of the definition of system boundaries. Finally, it feeds future work on reducing methodological biases and implicit assumptions in AESA.

5.07.P-Th206 Multi-dimensional allocation model within the AESA framework with Uncertainty

Propagation: a case study on the EU-27 automotive sector

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Absolute Environmental Sustainability Assessment (AESA) offers a science-based framework to evaluate whether human activities remain within the planetary boundaries (PBs).

This is done by downscaling the Safe Operating Space (SOS) -the space human activities can operate within to keep the environment similar to the Holocene conditions, which have allowed humankind to thrive- to a country/sector level by using predefined allocation principles. Yet, most AESA studies adopt a single, uniform sharing principle, typically population, to downscale global boundaries, neglecting both the heterogeneous nature of environmental mechanisms and the uncertainty embedded in sharing assumptions.

Here we developed a multi-dimensional (Multi-D) allocation model tailored to the biophysical and socioeconomic relevance of specific impact categories from the "EFv3.1" impact assessment method (PB-EF).

The developed approach aims at a more robust, transparent, and context-sensitive allocation approach for mitigating the limitations inherent in applying a single, uniform sharing principle across all categories, which can lead to biased outcomes (favoring some entities while negatively disadvantaging others).

We demonstrate this by applying this novel framework to the EU-27 automotive sector, focusing on passenger light-duty mobility to analyze how varying sharing principles and uncertainties influence absolute sustainability outcomes.

This work represents a crucial first step toward a unified methodology designed for the equitable distribution of global carrying capacities among countries, sectors, and companies.

5.07.P-Th207 PyUNCASE - Towards A Python Package for Systematically Allocating Carrying

Capacities Under Uncertainty in AESA

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The transformation of human activities to comply with carrying capacities must be supported by absolute environmental sustainability assessment (AESA) methods that allow stakeholders to compare their environmental impacts to their fair share of carrying capacities. The allocation phase has been identified in the literature as a major source of uncertainty in AESA results, as it can be grounded in various distributive justice theories and implementation options. Existing guidelines and frameworks for allocating carrying capacities (UNCASE) already constitute a robust scientific ground to systematically allocate carrying capacities in AESA, but they require specific knowledge, data, and time resources for practical implementation. In fact, UNCASE proposes probability-based AESA indicators by considering all available allocation methods together with other sources of uncertainty, such as uncertainty in life-cycle inventory data. Without a dedicated toolkit, the broad application of this framework remains limited, thereby relying on heterogeneous practitioner choices and implementations (with potential implementation errors, transparency and reproducibility issues). This leads to (i) arbitrary and limited choice of allocation methods in AESA studies, (ii) difficulty to properly interpret results, and finally (iii) difficulty to compare AESA outcomes across studies. This work hence presents the first python package (named PyUNCASE) for systematically allocating the carrying capacities in AESA. The open-source toolkit automatizes the assignment of carrying capacities down to the country and sector levels, considering available allocation methods. This allows practitioners to apply it systematically across countries and economic sectors. By considering available allocation methods in AESA, the proposed package supports ongoing discussions regarding the application of sharing principles. In addition, this offers a common starting point to promote harmonization in their implementation. The package also enriches current AESA guidelines by offering a flexible tool that can adapt to future consensus on carrying capacity allocation and new allocation methods. Its functionalities can be extended in the future (including the prospective allocation of carrying capacities and the calculation of probability-based AESA indicators by combination with LCA impact scores) to strengthen the robustness of AESA outcomes by supporting widespread uncertainty assessment.

Disclaimer/Disclosure:

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5.07.P-Th208 Assessing the Absolute Environmental Sustainability of Diets on a Spatialized Scale: A Case Study on Danish Tomato Consumption and Marine Eutrophication

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Global food production exerts significant pressure on the Earth system, contributing to the transgression of multiple planetary boundaries, particularly those related to biosphere integrity, land use, freshwater, and biogeochemical flows. Marine eutrophication is a highly localized impact category, yet current assessments rarely account for spatial differentiation or consumption-based perspectives. This study proposes a methodological framework for spatialized Absolute Environmental Sustainability Assessment (AESA) of food products, linking dietary consumption to local environmental boundaries. We apply the framework to quantify marine eutrophication impacts from tomato consumption in Denmark and compare these impacts to regional safe operating spaces (SOS) for oxygen depletion across coastal large marine ecosystem subsegments (cLMEss). Using national consumption data, origin-country trade models, spatial crop production allocation, life cycle inventories, and local characterization factors, we estimate impacts at a 5 arcmin resolution. Results reveal that production location strongly influences eutrophication impacts: although most tomatoes consumed in Denmark originate from Italy and Spain, the highest pressures occur in the North and Baltic Seas, where SOS transgressions reach up to 155%. In total, Danish tomato consumption affects ten cLMEss, including both transgressed and non-transgressed regions. These findings underscore the importance of regionalized assessments for localized impact categories and demonstrate the potential of spatialized AESA to identify impact hotspots. Extending this approach to full diets and additional impact categories can inform strategies for reducing environmental pressures through dietary shifts and procurement choices.

5.08 Increasing Access to Life Cycle Data: Bridging Transparent, Inclusive, and Reproducible Sustainability Knowledge

5.08.T-01 Toward Transparent and Inclusive Applications of Artificial Intelligence in Life Cycle Assessment

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Artificial Intelligence (AI), encompassing both machine learning and generative approaches, is increasingly explored in Life Cycle Assessment (LCA) to automate repetitive and data-intensive tasks such as mapping background processes. These capabilities could significantly improve access to life cycle data and foster reproducibility of LCAs. However, efficiency gains should not come at the expense of quality. AI introduces ethical and organisational challenges that may hinder transparency, fairness, and inclusivity among others, but existing reviews have largely focused on technical aspects, leaving these implications poorly explored.

This study critically analyses 150 AI-LCA applications (methods or tools employing AI to perform specific LCA tasks) identified through academic and grey literature. Applications were classified according to their origin and the LCA workflow steps they address. Each was evaluated using semi-quantitative scoring approach against 14 criteria grouped into four categories: technical maturity, potential to enhance LCA practice, operational feasibility, and ethical risks. Operational feasibility captures inclusivity by assessing ease of use, accessibility, and current adoption, while ethical risk criteria address fairness and transparency among others.

Results indicate that most applications focus on predicting impact score (50%), estimating missing data (30%), and mapping background processes (8%), with only a small fraction employing generative AI. While automation potential is high, most tools remain at proof-of-concept stage and lack full integration into LCA workflows. Operational feasibility is a major barrier: applications often require coding skills, lack open access, and provide limited documentation, resulting in low adoption. Ethical risks are significant, with low scores for fairness, transparency and accountability due to black-box models, insufficient documentation, AI divide, and representation bias in training data.

Although AI offers transformative opportunities for LCA, current practices fall short of the transparency and inclusivity required for responsible integration. Open-source tools with multilingual comprehensive documentation and intuitive interfaces, combined with guidelines to mitigate fairness, transparency, and accountability risks, should be developed for an inclusive, responsible and wide adoption of AI by the LCA community.

Disclaimer/Disclosure:

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5.08.T-02 Interoperability Requirements for Digital Product Passports in the Circular Economy

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The transition to a Circular Economy (CE) requires digital solutions that support industrial processes and increase transparency across the product lifecycle. The Digital Product Passport (DPP) is expected to play a central role, but its success depends on interoperability — the ability of systems and actors to reliably exchange and use data. This research, conducted within the EU-funded ENCIRCLE project, offers a structured analysis to guide the technical design of interoperable digital CE solutions.

A multi-stage methodological approach was applied. First, desktop research mapped the current regulatory and standardisation landscape, including the Ecodesign for Sustainable Products Regulation (ESPR), findings from the CIRPASS project and specifications of the Asset Administration Shell (AAS). Second, interviews with seven experts from industry, academia and standardisation bodies validated initial insights and highlighted

emerging trends. Third, an expert workshop with specialists in standardisation and AAS consolidated the requirements for CE use cases. The analysis followed the four dimensions of the New European Interoperability Framework: technical, syntactic, semantic and organisational interoperability.

The results show that the standardisation landscape for DPP interoperability remains fragmented, with existing standards covering only basic elements such as identifiers and secure data exchange. The work of CEN/CENELEC JTC 24 on core DPP standards and the ongoing standardisation of the AAS were identified as the most influential developments.

Across the four dimensions, clear requirements were derived. Technically, structured and openly documented REST-APIs are needed. Semantically, standardised data models, shared terminologies and globally resolvable identifiers are essential. Syntactically, machine-readable formats and mechanisms for data authenticity and integrity are required. Organisationally, governance structures and defined responsibilities for data quality are crucial. While blockchain was assessed as having low practical relevance, the role of AI for semantic mapping remains an open but promising topic.

This research provides a consolidated overview of the evolving interoperability landscape for the CE and supports developers, policymakers and industry stakeholders working towards data-driven circularity.

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5.08.T-03 Addressing the Reproducibility Problem in Life Cycle Assessment Studies: A Critical Scientific Challenge

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A growing reproducibility crisis is affecting many scientific disciplines, and environmental sciences, particularly Life Cycle Assessment (LCA), are no exception. Reproducibility has multiple and sometimes conflicting definitions. Here, we follow the US National Science Foundation's definition: "obtaining consistent results using the same input data; computational steps, methods, and code; and conditions of analysis." As reproducibility is a core scientific criterion; results that cannot be reproduced are unreliable claims. Despite recognition of this problem by LCA researchers and guidance documents offering recommendations, the reproducibility challenge persists in LCA. Reproducibility in LCA is hindered by inadequate reporting. Key modelling elements such as system boundaries, functional units, LCIA methods, and assumptions are often poorly communicated or omitted, while most inventories are reported in incomplete or non-standard formats. Reviews of hundreds of studies show that over 60% lack transparent inventory data.

ISO 14044 defines reproducibility as "a qualitative assessment of the extent to which information about the methodology and data values would allow an independent practitioner to reproduce the results reported in the study." This definition presents reproducibility as a spectrum, the closer the reproduced results are to the original, the higher the degree of reproducibility. Thus, it can be concluded that the list of information required to be reported by ISO is not sufficient nor meant to achieve bitwise reproducibility. This gap shows the need for clear, detailed guidance and accessible tools that enable practitioners with varying levels of experience to perform reproducible LCAs.

In this work, we conduct three complementary reviews to identify the gaps underlying the reproducibility challenge in LCA and to develop adoptable solutions. The first examines the LCA guidance documents, the second reviews LCA research, and the third reviews meta-LCAs. We then develop a documentation framework for achieving reproducible LCAs that relies on structured, systematic, and standardized reporting of all relevant methodological choices, inventory data, and LCIA results. We operationalize the framework with an intuitive spreadsheet-based tool. This framework renders environmental impact results scientifically testable despite confidentiality constraints and facilitates meaningful meta-analyses by making the spreadsheet template machine readable.

5.08.T-04 PROXYLCA : A Standardised Framework for Estimating Missing Food Life Cycle Inventory Data

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Global food systems account for roughly one-quarter to one-third of anthropogenic greenhouse gas emissions, occupy about half of the world's habitable land, and consume over 70% of global freshwater, with most of these impacts occurring at the production stage. As countries increasingly depend on food imports, trade patterns have become a key driver of these pressures. However, the limited geographical coverage of existing life cycle inventory (LCI) databases—heavily focused on Europe and North America—makes it challenging to assess the sustainability of global food trade patterns, particularly from Latin America, Africa, and Asia which are acutely affected by agri-food resource extraction. Current extrapolation methods to fill these gaps often require extensive data and fail to accurately transpose detailed field-level emission modeling embedded in the original inventory, limiting their reproducibility and scalability.

To address this gap, we developed PROXYLCA, a standardised, globally applicable framework that uses multivariate similarity analysis of large agricultural databases (FAOSTAT) to identify suitable proxies in LCI databases. The framework constructs composite proxies of existing LCIs without altering original emission models, regionalises the proxy LCIs to reflect local conditions, assesses data quality using a matrix-based approach, and provides recommendations on using principal component analysis to interpret results. Applied to 230 product–country combinations across 59 countries, PROXYLCA enables comparisons of environmental performance between imported and domestically produced foods. For approximately half of these combinations, existing LCIs allowed validation of the framework and results. Initial findings highlight regional differences in impact categories such as water scarcity and biodiversity, underscoring the importance of regionalisation.

PROXYLCA offers a practical and scalable solution to fill food LCI data gaps, supporting transparent and reproducible life cycle assessments which can help guide sustainable trade policies on the basis of robust, geographically relevant impact assessment.

5.08.P Increasing Access to Life Cycle Data: Bridging Transparent, Inclusive, and Reproducible Sustainability Knowledge

5.08.P-Tu430 Scaling Industrial Life Cycle Sustainability Assessments through Automation and Standardization: An Assessment of Barriers and Technical Solutions

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Integrating sustainability metrics into routine industrial decision-making remains challenging because current Life Cycle Assessment (LCA) practices are often too complex and inconsistent to allow for standardized industry adoption. These challenges are becoming more urgent as CO₂ pricing mechanisms (e.g., EU ETS, CBAM) create direct financial consequences for industrial sectors. Comprehensive sustainability assessments that integrate social and economic dimensions of sustainability, such as Life Cycle Sustainability Assessments (LCSA), introduce yet additional challenges. This motivates the following research questions:

1. What are the current barriers that limit the wider adoption of LCA for industrial applications?
2. What additional challenges does integrating economic and social assessments, as part of the LCSA methodology, present – especially for industry application?
3. Most important: Which opportunities exist for targeted standardization and computational automation to enable scalable, reproducible, and comparable sustainability assessments for industrial sectors?

Drawing on a structured review of existing challenges, we develop a research framework that organizes barriers into categories, such as data scarcity, methodological inconsistency, and low applicability of results, and identifies opportunities where advanced computational tools could provide substantive improvements. The framework highlights technical solutions such as Machine-Learning-supported data mapping, automated process decomposition using Large Language Models (LLMs), integrated uncertainty modeling, and multi-criteria trade-off analysis. For each opportunity, we assess its potential impact, such as expected cost and time savings, its limitations, and its implementation feasibility.

Our analysis suggests that combining targeted methodological standardization with computational automation

can significantly reduce assessment uncertainty, improve reproducibility, and enable a shift from retrospective compliance toward proactive decision support. This study contributes a comprehensive synthesis of current obstacles and provides a framework that defines strategic opportunities to accelerate the industrial adoption of LCSA practices.

5.08.P-Tu431 Data Based Buildings Archetypes for Probabilistic LCA

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Context - To reduce the environmental impact of urban development, planners must assess proposed construction at the earliest design stage. At this stage, urban projects are defined only by programmatic areas, and planners typically lack information about building geometry or material composition. Archetypes thus provide a solution for urban scale Life Cycle Assessment (LCA). However, current studies often use a single archetype for all buildings uses and most of them omit uncertainty and sensitivity analyses, even though the main goal of such early assessments is to determine both a mean value and the variability—the range of the projected construction impacts. In this work, focusing on materials, we investigate different methods for constructing program-specific archetypes (Individual housing, Collective housing, Offices, etc.), enabling comprehensive LCA (uncertainty, sensitivity and contribution analyses) of urban projects thanks to a French buildings database.

Methods – Common construction products processes are created to link the archetypes to a background database (ecoinvent). Two methods are then applied to the French Buildings Regulation ('RE2020') Observatory database to create program-specific archetypes. The first, direct strategy defines each archetype as a weighted linear combination of all buildings belonging to a given use category. A second approach use pre-calculated materials intensities to link the archetypes to the created products-processes. Along the way, we introduce a strategy to deal with lognormal fitting and lists of values that contain many zeros.

Results and Discussion – Results for both kind of archetypes are discussed. The first approach appears to be time-consuming but reliable, whereas the second is faster but introduces unrealistic products combinations, creating outliers that greatly increase standard deviations. This preliminary study shows that program-specific archetypes can be created from built projects database and employed in a probabilistic LCA. It also highlights that a more transparent buildings database with accurately identified construction products could reduce the uncertainties on LCA results. Future work will improve these initial results : computational efficiency of buildings-based archetypes will be increased through reused Monte-Carlo runs on products, and the physical relevance of materials intensities based archetypes will improved by introducing dependant sampling.

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5.08.P-Tu432 From Parametric LCA to Digital Twin: a Model and Data Framework for LCA

Automation

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LCA is essential for evaluating environmental performance, yet traditional approaches are often static and labor-intensive, limiting their use in dynamic systems where variations in operating conditions demands models that are flexible and automated. At the same time, industry is embracing digitalization through real-time data, automation, and digital twins—virtual representations of physical systems. Integrating LCA into these frameworks enables dynamic, data-driven sustainability assessments. This study outlines the development of a parameterized LCA model designed to incorporate both data variability and process variability within the assessment framework, while enabling automation of calculations. The application context is microalgal production, a process characterized by high variability due to factors such as meteorological conditions, geographical location, and operational capacity.

Given this variability in operations, it is essential to adapt the LCA model not only in terms of the input values used for modeling but also in terms of its structural configuration—specifically, the types of activities included under different operational scenarios. To address this challenge, we developed data collection guideline tailored

for the development of a parametrized and digitalized LCA model. This guideline is tailored to accounts for variability effects, prioritizes components already utilized in production, and identifies specific sources of variability within the system, that are then turned into model parameters. Next, interviews with technology developers clarified the current level of digitalization and data integration, so that the LCA model can build on top of existing capacity and seamlessly integrate with that. The parameterized model is then integrated with a digital twin representation of the production facility, thereby enhancing dynamic assessment and decision-making capabilities. A semantic schema then maps primary process information to the provided secondary data and elements of the LCA model and allows for automated reasoning and model reconfiguration. The implementation of the data mapping and the interface to an LCA software (brightway) is described through a functional and logical system architecture. Overall, this system allows an automated LCA to be implemented via a software-based microservice that can be integrated into existing industrial IT environments through appropriate communication endpoints.

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5.08.P-Tu433 An Innovative Method for Retriving Inventory from Product Definition: LCA of Magnesia-carbon Refractory Ceramics

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Refractory materials are essential for sustaining high-temperature industrial processes, with the steel industry being the largest consumer. Collecting inventory for refractory production is challenging due to confidentiality concerns and suppliers' reluctance to share data. To overcome this, an innovative method has been developed to retrieve essential inventory data from the refractory's product definition. The method is demonstrated through a case study comparing two magnesia-carbon refractory bricks used for steel ladle slag lining: one produced in China and the other in the Netherlands. Inventories for brick production are derived from the respective product definitions and compared actual industrial data. Life cycle assessment results reveal that the Netherlands brick performs better in climate change and acidification, while the Chinese brick shows lower impacts in other categories considered in this study. Electricity consumption during magnesia production and thermal energy demand for ladle heating are identified as the major environmental hotspots. Energy transition scenarios demonstrate the potential for significant improvements. Replacing national grid electricity and natural gas with renewable electricity and green hydrogen leads to more than 30% reductions in most impact categories. Climate change impacts, for instance, decrease from 1.89 to 0.97 kg CO₂ eq for the Chinese brick and from 1.67 to 0.77 kg CO₂ eq for the Netherlands brick. Sensitivity analysis shows that magnesia production strongly influences environmental performance, while uncertainty associated with product-definition-derived data is generally minor for most impact categories.

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5.08.P-Tu434 Multi-regional Chemical Emission Inventory for Freshwater Ecological Risk based on PRTR and GLORIA

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Freshwater ecosystems face a biodiversity crisis: roughly one quarter of species are at risk of extinction, largely due to chemical pollution and agricultural activities. The Global Framework on Chemicals (GFC) calls for evidence-based management of chemicals across their life cycle. In LCA study, impact assessment methods such as the USEtox model and the use of hazard concentrations for 20% of species (HC20) have advanced. On the inventory side, Leclerc et al. (2023) estimated a global toxic footprint by combining multiple emission

databases with an environmentally extended multi-regional input–output model, but their assessment relies strongly on extrapolation using proxy indicators (e.g., economic output) where Pollutant Release and Transfer Register (PRTR) data are lacking, leaving room for more detailed analysis of country- and sector-specific emission patterns.

This study aims to develop a multi-regional chemical emission inventory by integrating national PRTR data with the Global Resource Input–Output Assessment (GLORIA) database and to link this inventory to freshwater ecological risk assessment.

We first selected approximately 200 chemicals of concern for freshwater ecosystems, including metals, from PRTR in regions such as Japan, Europe, North America, and so on. Facility-level annual releases were then compiled by compartment (air, water, soil) and industrial activity, and each emitting facility was mapped to the corresponding GLORIA sector to calculate emissions by country, substance, and sector. For countries and substances without PRTR coverage, we completed the inventory by combining sectoral production data from GLORIA with emissions derived from PRTR countries. For ecological risk assessment, we applied a nonlinear model for algae, invertebrates, and fish, used species sensitivity distribution curves to describe log-normal concentration–effect relationships for each substance, and derived characterization factors by accounting for mixture toxicity across multiple substances and species.

This presentation reports initial analyses of emission patterns and examines how key inventory assumptions influence ecological risk indicators. The integrated PRTR–GLORIA inventory provides a promising approach to explicitly represent regional and sectoral differences in chemical emissions within LCA and can support the identification of chemical pollution hot spots, priority substances, and options for improving national chemicals management policies.

5.08.P-Tu435 Towards Reproducible, Transparent, and Interoperable LCI: the Role of Collaborative Digital Tools

Gabrielle Tihon, Martin Besnier, Paul Grédigui, Alice Marchal, Yohan Lanier, Mathis Girard, Eulalie Bonnot and Antoine Bouvart, Holis, France

Context

As sustainability assessments become central to regulatory and corporate decision-making, the demand for comprehensive and specific Life Cycle Inventory (LCI) data is rapidly increasing. Yet, LCI data collection remains fragmented and largely confined to conventional LCA-oriented sources, overlooking complementary sustainability data such as Material Flow Analysis, logistics data, or risk and compliance information. This lack of integration limits data completeness, traceability, and reproducibility, especially across global supply chains.

Research Question

How can a digital, collaborative data collection system improve the completeness, reproducibility and interoperability of LCI data by integrating heterogeneous sustainability data sources, while engaging supply chain stakeholders in continuous improvement?

Methods

We propose a collaborative data collection tool - Holis Workshop - aiming to simplify seamless data exchange between brands and suppliers, while adding value for both parties. Holis Workshop works together with Holis Studio - LCA & eco-design platform - but can virtually be paired with any LCA software.

The tool allows standardized and flexible data requests at process, product, and portfolio levels, while connecting with LCA software and enterprise systems (ERP, PLM). Its architecture is designed to integrate non-traditional LCI data sources (e.g. logistics datasets, material flow data) through common data structures and mapping protocols to ensure data reproducibility.

Advanced features under development include automated anomaly detection, consistency checks, and impact-driven prioritization of data gaps, improving data robustness and uncertainty management.

For suppliers, the tool reduces data sharing burden through existing systems integration (ERP, PLM) and efficient batching. In future versions, the tool will allow suppliers to value all transmitted data and offer cost-saving insights and eco-design recommendations on their own processes. We are actively gathering industry feedback to ensure this feature meets real-world needs and expectations.

Conclusion

Holis Workshop demonstrates how collaborative digital infrastructures can bridge fragmented sustainability data ecosystems and move beyond conventional LCA data boundaries. By integrating diverse data sources and enabling transparent, reproducible, and scalable LCI data collection, the approach contributes to more robust inventory modeling, improved regulatory alignment.

5.08.P-Tu436 A Data Structure for Life Cycle Inventory and Life Cycle Assessment Data in the Digital Product Passport

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Digital Product Passports (DPPs) are a tool for digitally transferring information along value chains. DPPs can support Life Cycle Assessments (LCAs) by improving data availability and reducing the burden of data collection along supply chains. Particularly, embedding life cycle inventory (LCI) data within DPPs has the potential to enhance the transparency and standardisation of product sustainability reporting. However, the practical integration of LCI and LCA data into DPPs raises methodological and structural questions regarding how environmental information should be collected, organised, and communicated in this context.

In this paper, we investigate potential pitfalls and their solutions in the incorporation of environmental footprints in DPPs. We outline data requirements for PEF-compliant LCA studies, both for output data (PEF results) and input data (unit process LCIs and product system parameters), and translate these to a DPP system. From this, we derive a comprehensive data structure for unit process LCI data (inputs) and LCA results (outputs) in DPPs that enables standardisable, PEF-compliant environmental impact calculation.

We demonstrate the relevance of this data structure using two case studies: printer cartridges and heat pumps. By comparing and contrasting applications across these different product categories, we aim to show the general applicability of our data structure. We conclude with recommendations for the implementation of sustainability indicators in DPP regulations and infrastructure.

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5.08.P-Tu437 Integrating Life Cycle Data into Digital Product Passports: Open-Source vs Licensed Inventories

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Digital Product Passport (DPP) is an emerging instrument that may enable greater access, transparency, and traceability of product sustainability and life cycle data across supply chains. According to current European Commission proposals, DPPs will include Life Cycle Assessment (LCA) inventory data and impact assessment results, making the availability of accessible and high-quality datasets a critical prerequisite. However, widely used LCA databases may be subject to restrictive licenses, limiting their future integration into DPPs.

To address this data-access challenge, the study explores whether open-source LCA inventory datasets can support DPP implementation without compromising methodological robustness. To answer this research question, an exploratory case study focused on laser welding processes is used as a test bed. Because laser welding represents an intermediate manufacturing step used across multiple value chains, its inventory data can directly support the DPPs of diverse final products, including pharmaceutical devices, automotive components, and battery systems. Specifically, the study compares the modelling of this process using licensed ecoinvent data and other open-source alternative databases.

The study aims to map differences by evaluating inventory flows, system modelling consistency, and data completeness under harmonised LCA assumptions. Expected results include the identification of key structural and methodological differences between databases. These may relate to the sector-process hybrid versus the process-based structures, as well as to the emphasis on carbon footprint information compared with broader product footprint data.

Beyond the specific laser welding case, the study emphasizes the broader relevance of improving access to life cycle data as a foundation for effective DPP implementation. In this context, achieving an appropriate balance between the openness of publicly available inventories and the methodological robustness and the granularity of licensed datasets such as ecoinvent emerges as a key challenge across all product sectors. The insights generated aim to support policymakers, database developers, and industry stakeholders in facilitating the reliable integration of LCA data into future DPPs, ultimately contributing to more transparent and interoperable digital product information systems.

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5.08.P-Tu438 Reusables, Disposables and Systems In-between – A Comparison of the Environmental Impacts of Trocar Systems Using Life Cycle Assessment and Material Characterization

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While healthcare is important, it is also responsible for approximately 7% of national greenhouse gas emissions and 4.2% of all waste in the Netherlands. One component that contributes to this issue is the usage of a lot of disposable instruments, such as the trocar. This instrument that is used in all laparoscopic surgeries to create an opening into the body is available as a disposable, reusable and hybrid system. These different systems all have their own perceived advantages, one of which is that the reusable system is often assumed to have the lowest environmental impact. However, the environmental impact of a trocar system depends on several factors, such as the specific type and the hospital. This research therefore not only determines which trocar has the lowest impact, but also what other variables involved at the hospital lead to more sustainable practices. This is done by analyzing different types of trocars in different settings. The environmental impacts were calculated through life cycle assessment using Activity Browser and Ecoinvent (cut-off). The scope for all different systems was cradle to grave and excluded hospital infrastructure and any potential repairs of the trocar systems. To consider all different variables, data was gathered directly through material analysis (more specifically, Fourier-Transform Infrared Spectroscopy), interviews and surveys where possible. Preliminary results reveal that the reusable trocar system used in the academic hospital with external sterilization had the lowest carbon footprint (0.32 CO₂eq) whereas usage of the disposable system at the same hospital led to the highest impact (1.74 CO₂eq). The impact of the former mainly originated from the sterilization process (91%), whereas the latter was mostly caused by the manufacturing phase (81%). These results can subsequently be used to define other points of improvement to reduce environmental impacts. The findings can help to make all laparoscopic surgeries more sustainable. Furthermore, the research provides a workflow that can also be applied to other medical instruments to continue decreasing the impact of the healthcare sector.

5.08.P-Tu439 Life Cycle Insights of Regional Natural Gas Liquids (NGLs) Supply and Use as Steam-Cracker Feedstock

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The steam cracking process supplies essential building blocks used in the chemical and plastic industries, namely light olefins and aromatics. Heavy hydrocarbons remain a dominant global feedstock for steam cracking. On average, such feedstock mixture consists mainly of naphtha and atmospheric gas oil accounting for about 51% and 6%, respectively. At the same time, natural gas liquids (NGLs), whether as mixed streams or separated fractions (e.g., such as ethane, propane, and butane), are gaining importance as feedstock for steam crackers worldwide.

Notably, supply chain models used in life-cycle databases often treat NGL feedstocks and downstream steam cracking systems as regionally homogeneous. This approach, however, neglects substantial regional differences in upstream extraction, fractionation, transportation, and steam cracking feedstocks and facility configurations, which can significantly influence energy demand, greenhouse gas emissions, and other environmental burdens.

Versions 3.11 and 3.12 of the ecoinvent database provide enhanced geographic coverage of oil and gas extraction for 40 countries, natural gas supply mixes for 57 countries, refined regional transport and pipeline/LNG shipping distances. Building on this geographical representation, we present how regional NGL production and fractionation affect the life-cycle impact assessment (LCIA) results of key feedstocks and how these effects carry through regional steam cracking operations.

Developed within ecoinvent, we present a comprehensive LCI modelling framework that enables capturing the regional variability of NGLs production/fractionation in key producing countries. By applying region-specific parameterization instead of a one-size-fits-all assumption, we reveal substantial variation in LCIA results. For instance, regional carbon-intensity scores for ethane from NGL fractionation range from 50% below to 11% above the global average representation.

This analysis underlines the importance of adequate supply chain resolution: from NGL extraction via fractionation and transport to steam cracking operation regions, for more credible LCIA results for key

hydrocarbon feedstocks and petrochemicals. Looking ahead, the ecoinvent team will expand the modelling beyond steam cracking to enable geographically explicit supply chains from oil and gas extraction to NGL production. On this basis, further regionalizing downstream petrochemicals and plastics production remain a key objective.

5.08.P-Tu440 Beyond Standard Distances: Modeling Transport Routes in the ecoinvent Database

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Transport accounts for 23% of global energy-related CO₂ emissions, yet LCA databases like ecoinvent generally rely on simplified distance assumptions. Current methodologies often lack the integration of world trade flows, distance-calculation algorithms, and transparent uncertainty characterization; these gaps become critical as regulatory frameworks demand greater data specificity.

With the aim of improving the accuracy and transparency of transport distances in ecoinvent's inventories, we present a novel methodology that incorporates global trade and transport data into the ecoinvent database. The approach identifies production and consumption centers within countries, then computes origin and destination hubs through clustering and optimization algorithms, ultimately connecting these hubs into a network.

UNCTAD trade statistics provide empirical modal splits for the different segments, supported by mode-specific solutions that are used for routing and distance calculation: OpenRouteService for road and rail, the Python searoute package for maritime, and great circle distance for aviation. Benchmark validation against real-world data confirms the accuracy of the methodology: results fall within a 0.5-5% interval across different modes of transport.

The results provide distance calculations for different product categories differentiated by fleet composition, emission standards, and regional characteristics. This improves the distances and the related emissions used in the ecoinvent database while providing transparency on underlying data and modeling. The framework demonstrates how data integration, transparent validation, and AI-supported automation can transform static estimates into dynamically calculated and realistic transport distances. Overall, this new approach gives a more accurate representation of the impact of transport in supply chains.

5.08.P-Tu441 Describing the development of harmonised life-cycle inventories for Brazilian pasture systems supporting beef production.

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This study describes the development of harmonised life-cycle inventories for Brazilian pasture systems supporting beef and dairy production. A stepwise process was implemented combining literature review, inventory construction in ICV Calc Software, export and refinement in LCA software, and expert validation through workshops. Three pasture management systems (extensive, semi-intensive and intensive) were characterised, with explicit documentation of inputs, operations, and system boundaries both for beef and dairy cattle, totaling 25 inventories combining distinct pastures and management systems. Scenarios with and without land use change (LUC) were generated using the BRLUC methodology. A comprehensive screening of scientific studies enabled benchmarking and ensured methodological transparency. This iterative workflow produced a set of traceable and replicable pasture datasets, laying a qualitative foundation for future quantitative analyses, literature comparisons, and low-carbon beef certification programmes.

5.08.P-Tu442 New modelling approach for multi-nutrient inorganic fertilizers in the ecoinvent database: solving the double-counting issue.

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In ecoinvent database, multi-nutrient inorganic fertilizers (MNIFs) can be modeled either as a composition of single inorganic nutrients (SIN) (as N, P₂O₅, K₂O) or as complete fertilizer products (such as NPK (15-15-15)). The supply chain starts from: i) The chemical production activity of MNIFs linked to their market; ii) Markets are connected with nutrient activities supplying the SIN of MNIFs; iii) The nutrient supply activity is an intermediary between the chemical production and the market mix of SIN; iv) The market mix providing SIN from the MNIFs to the agricultural production systems.

When MNIFs are applied in crop production datasets, the amounts of SIN are partially double-counted or, in some cases, underestimated due to the supply chain. The new modelling addresses this issue by estimating the

residual nutrient contribution dose (RNCD) from MNIFs and applying correction flows at the crop inventory level. The RNCD of each SIN from the MNIFs is calculated based on the nutrient composition of MNIFs per the exchange amount in the crop dataset. As a result, the inorganic fertilizer is now linked to the MNIF for each specific SIN (e.g., “inorganic nitrogen fertilizer, as N, from NPK (15-15-15)”). Finally, market datasets for MNIF fertilizers are recalculated to rebalance nutrient shares, ensuring that the quantities reflect the compositional ratios of each MNIF. The new modeling affects approximately 400 datasets. The results show a reduction (or slight increase) in LCIA scores in most cases, since inorganic fertilizers are among the most impactful inputs in agricultural inventories.

5.08.P-Tu443 Data Extrapolation in Life Cycle Inventories for the Agri-food Sector: Challenges and Approaches

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Life Cycle Assessment (LCA) has become an essential tool for evaluating the environmental impacts of the agri-food sector, yet researchers often encounter data gaps in life cycle inventories (LCI), especially when analysing region-specific practices, emerging food products, processing technologies, or situations where primary data collection is not feasible. Inventory data extrapolation becomes a crucial methodological necessity, but it also introduces significant uncertainties that can compromise the robustness and reliability of LCA results. Accurate and representative inventory data are crucial for reliable LCA studies, as the quality of LCI directly affects the robustness of impact assessment results and, consequently, the credibility of sustainability conclusions. When primary data are incomplete, missing, or inconsistent, extrapolation allows practitioners to estimate, adapt, or extend available information to develop more comprehensive inventories. However, the range of extrapolation approaches and the lack of harmonised guidance present challenges for methodological transparency and comparability, especially in agri-food systems where processes vary significantly across regions and production conditions.

This work conducts a structured literature review to examine the role of data extrapolation in LCI development for the agri-food sector. The review aims to identify and discuss the main types of data gaps reported in agri-food LCAs, exploring challenges and sources of uncertainty within the sector, list common extrapolation practices used in the context of LCIs, and identify strategies to improve the reliability and transparency of extrapolated data. Approaches such as proxy datasets, scaling methods, and contextual adjustments are examined regarding their potential advantages, limitations, data requirements, and suitability for different gap scenarios. It also investigates how these practices influence uncertainty and how their applicability varies with technological, geographical, and temporal characteristics of the agri-food sector systems.

This work advances LCA methodology by critically analysing extrapolation practices in agri-food contexts and suggesting ways to improve transparency, reproducibility, and reliability when data gaps require extrapolation in LCA development.

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5.09.A Life Cycle Assessment for Sustainability Decisions: Methodological Challenges and Future Directions

5.09.A.T-01 People in the ISO Life Cycle

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When the generic standards for life cycle assessment (LCA; ISO 14040 and 14044) are due for systematic review next time, the arguments for revising the standards are strong. Both clarifications and updates are needed. However, increasing the clarity of the standards will be a major challenge, because long-standing conflicts over the LCA methodology remain – for example on allocation and the choice of input data. Resolving these conflicts probably requires constructive discussions based on a deeper understanding of the opposing positions. The aim of this presentation is to contribute to such understanding. We explore how different mental models of the life cycle can relate to different ideas of the purpose of LCA and, hence, to different opinions on

the appropriate methodological choices. We do so by describing two archetypical examples of LCA experts.

“The chemical engineer” considers the life cycle as a system of physical flows – an extension of the flow charts used in process industry. The task of an LCA practitioner is then to quantify these flows as precisely as possible. Input data on activities in the supply chain should ideally be primary data from the contracted suppliers. Marginal, credit-based mass-balance and book-and-claim approaches are problematic because they result in models that deviate from the physical reality. Substitution can be problematic because it increases uncertainty and, hence, reduces precision in the results. Economic allocation is avoided because it is not robust over time. When an allocation problem occurs, it is instead reasonable to look for the best physical basis for the allocation.

“The systems scientist” observes that the purpose of LCA to assist in the transition to a sustainable society and defines the life cycle as a large sociotechnical system. Human decision-makers, market mechanisms, etc. are important aspects of this system. Relevance for decision-making and incentives for change are more important than precision. Consequential LCA is relevant because it takes market mechanisms into account in the use of marginal data and substitution. People can be accounted for also in attributional LCA through economic allocation. Chain-of-custody approaches should be assessed case by case depending on whether they drive change or merely allow for greenwashing.

Constructive debates on the mental models and on the purpose of LCA can lead to better understanding of opposing positions on the methodology.

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5.09.A.T-02 Measuring Sustainability and Circularity of Products: Development of a Harmonized Digital Material Passport for Chemicals Enabling the Transfer of LCIA, Circularity and Safety Data Along Supply Chains

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The transition toward a circular economy and the implementation of sustainability principles in product design require transparent, interoperable, and verifiable data exchange across complex supply chains. Upcoming regulatory frameworks in the European Union and other jurisdictions mandate Digital Product Passports (DPPs) for diverse product categories, including textiles, tyres, construction materials, and detergents. These developments will significantly impact chemical manufacturers, who must provide cradle-to-gate life cycle impact assessment (LCIA) data, circularity metrics, and product safety information (e.g., substances of concern) to downstream sectors in a standardized and scalable manner.

This contribution outlines an approach for developing a harmonized Digital Material Passport (DMP) for chemicals, designed to enable the efficient transfer of sustainability and circularity information along value chains. The work includes sectoral specific guidelines for environmental sustainability, circularity, and material declaration grounded on ISO/EN standards and landmark regulations and frameworks, complemented by an emerging information model that defines a comprehensive set of attributes for LCIA and circularity metrics, as well as material declaration. These elements form the foundation for a horizontally harmonized vocabulary and determination methodology, ensuring consistency across sectors and alignment with regulatory requirements. The proposed framework addresses three critical challenges:

1. Data interoperability through standardized vocabularies and machine-readable formats;
2. Methodological harmonization for calculating LCIA and circularity indicators at the chemical product level;
3. Integration into digital ecosystems to support secure, scalable data exchange between upstream and downstream actors.

Preliminary results demonstrate how harmonized guidelines and attribute models can reduce complexity and foster transparency, enabling chemicals to serve as enablers of sustainability in downstream applications. The presentation will discuss the developed LCIA and circularity framework, as well as the conceptual architecture of the DMP, key design principles for interoperability, and lessons learned from initial implementation efforts. This work contributes to accelerating compliance with DPP regulations and advancing the circular economy in the chemical sector downstream applications.

5.09.A.T-03 The EcoBeautyScore: A Science-Based and Harmonized Environmental Scoring Scheme for Cosmetics

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The EcoBeautyScore (EBS) Association has created a harmonized, science-based framework to measure and communicate the environmental performance of cosmetic products. Built on Life Cycle Assessment (LCA) and aligned with the European Commission's Product Environmental Footprint (PEF) guidelines, the methodology adapts PEF principles to the specific characteristics of cosmetics, considering their diverse functions and usage patterns. Since mid-2025, the system has been progressively deployed in Europe to support transparent consumer communication.

The footprinting approach evaluates 16 environmental impact categories across the full product life cycle, using PEF-consistent normalization and weighting to obtain a single aggregated footprint. To ensure comparability, products are grouped into 30 coherent segments defined by their primary function, each with standardized functional units and default usage doses.

A major pillar of EBS is the construction of harmonized ingredient databases covering production and end-of-life impacts. Due to the large diversity of cosmetic ingredients, a prioritization strategy was applied to identify the most impactful and frequently used substances. Data were sourced from public datasets and industry contributions; when needed, scientifically robust default values were used. Significant advances were made in ecotoxicity modelling, especially to address limitations in USEtox 2.1. The Most Sensitive Species–HC5 approach was adopted to improve the robustness and coverage of effect factors.

To support consumer understanding, the aggregated footprint is translated into an A–E score. This scoring relies on segment-specific statistical calibration based on a representative sample of at least 100 marketed products per segment, ensuring realistic and meaningful thresholds.

Testing on over 1,900 products revealed strong differences between product types. For rinsed-off products, the use phase dominates impacts but offers little differentiation; ingredient production and end-of-life phases are more discriminating. For leave-on products, packaging and ingredient end-of-life are the main contributors. These findings highlight the importance of refined ecotoxicity modelling and segment-specific assessment. Overall, the EBS framework provides a robust, scalable, and transparent system to assess and compare the environmental impacts of cosmetic products, supporting both consumer information and eco-design strategies.

5.09.A.T-04 Advancing Consumption-Based Decision Support: Modelling Carbon and Biodiversity Border Taxes for EU Food Systems

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The European Union's food system exerts disproportionate pressure on global climate and biodiversity, largely through imports from high-impact regions. To support evidence-based decision-making on sustainable food systems, we evaluate how trade-based policy measures can reduce the environmental footprint of EU food consumption. We integrate a high-resolution, multi-regional input–output framework, spatially explicit land-use-change emissions, ecoregion-level biodiversity risks, and EU-specific demand elasticities to assess the effects of carbon and biodiversity Border Adjustment Taxes (BATs) on the EU's international food supply chains. We model carbon, biodiversity, and combined BATs across ten tax levels, and assume that high-impact imports are replaced with domestic EU production. Results show that a combined BAT of 100 €/tCO₂-eq and 100 × 10¹² €/PSL_{glo} reduces EU food-related impacts by 9% (climate) and 15% (biodiversity), with even greater reductions for beef (–12% climate, –35% biodiversity). Reductions are concentrated in Latin America, Africa, and Asia–Pacific, and are accompanied by job losses and value-added shifts in exporting regions. A small number of trade flows drive much of the effect: the Netherlands' and Italy's reduced beef imports from Brazil, Australia, Namibia, and Botswana account for half of the environmental impact reduction of beef, while France's reduced imports of vegetables, fruits, and nuts from Morocco and Côte d'Ivoire account for a quarter of total job losses. While BATs alone cannot align EU food consumption with planetary boundaries, they provide strong leverage for targeting high-impact flows. Integrating carbon and biodiversity pricing into EU trade policy would fill a critical gap and advance the Green Deal and Farm-to-Fork objectives. This study supports policymakers by identifying leverage points in international supply chains and quantifying the global consequences of sustainability-oriented trade measures.

5.09.B Life Cycle Assessment for Sustainability Decisions: Methodological Challenges and Future Directions

5.09.B.T-01 Applying Life Cycle Assessment at Different Levels: Deriving Insights for the Potential of Bio-based Solutions

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The scarcity of natural resources and increasing environmental pressures due to current production and consumption patterns demand policies to ensure the EU's competitiveness and sustainability. The bioeconomy offers a pathway to transition towards greener systems. Life Cycle Assessment, a method for evaluating environmental impacts across the whole value chain and life cycle stages, enables analysis of multiple environmental impacts, unveiling burdens, benefits and trade-offs, but at the same time facing challenges in comparability between studies due to varying system boundaries and assumptions. LCA can be used to assess and compare the environmental performance of products, as well as to allow analysis at a more aggregate level. The aim of the study is to assess environmental impacts using illustrative case studies at the material (e.g., plastic packaging or fuel), and product level (e.g., packaged product or vehicle), up to enlarging the analysis to selected insights at the EU aggregate consumption level, both as a total consumption of selected materials and when considering the average EU citizen. By analyzing impacts at these scales, the study reveals insights into material choices, product performance, and broader consumption trends, supporting informed decision-making for sustainable development.

The magnitude of environmental impact differences diminishes as the assessment shifts from the material to the product level, and further to the EU citizen level. This variation depends on modeling assumptions, the type and relevance of the conventional material, and the feedstock of bio-based alternatives. Nevertheless, the results provide valuable insights on the expected environmental impacts, trade-offs, and key leverage points in consumption that support the transition to sustainability. At the material level, the choice of bio-based materials is relevant for specific environmental impacts. Instead, product level analysis highlights leverage points and potential benefits across the entire value chain. The benefits obtained at the EU level depend on the rate and share materials can substitute their conventional counterparts. Results also show the importance of conducting analysis at various levels while transparently reporting all modelling choices and assumptions behind, to make sure correct and consistent messages are conveyed to support the research and policy debate in finding solutions for sustainability transitions.

5.09.B.T-02 Enhancing Comparative Life Cycle Assessment Decision-Making Through Objective Reduction of Impact Categories Under Uncertainty

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Comparative Life Cycle Assessments (LCAs) often include many midpoint impact categories, which can make results harder to interpret and decisions more complicated. Existing methods for choosing relevant categories were developed for single systems and are not well suited to comparative studies that involve uncertainty. This research aims to develop an objective and transparent way to reduce the number of impact categories while keeping results meaningful and reliable. The main hypothesis is that if the difference between systems is neither statistically nor practically significant, that category may not be considered for decision-making.

The proposed framework focuses on the Life Cycle Impact Assessment (LCIA) step and integrates uncertainties in characterization factors, which are often simplified or ignored in current studies. These uncertainties are represented through probability distributions and analyzed using statistical tests. A combination of significance testing and effect size analysis helps identify which categories show real and meaningful differences. Depending on the comparison, paired t-tests or one-way repeated-measures ANOVA are used, with corrections applied to avoid false positives and prevent type-1 errors. Only categories showing statistically and practically significant differences are selected for decision making.

The approach was tested by comparing the production of one kilogram of three lithium-ion battery chemistries, LFP, NCA, and NMC111 using data from ecoinvent 3.11 and the EF 3.1 method. Uncertainties of twelve impact categories were analyzed using 1,000 samples of characterization factors generated through Latin Hypercube Sampling. The results showed that six categories had both statistical and practical significance, confirming the method's ability to simplify comparisons by objectively reducing the number of impact categories.

This framework provides a clear, data-driven approach to support decision-making in comparative LCAs. It can be applied to any LCIA method, depending on the availability of uncertainty data, and remains flexible to meet user-specific needs. Future work will extend the framework to a territorial context, connecting results to

planetary boundaries to evaluate how close each impact is to critical environmental thresholds and to further refine the selection of impact categories.

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5.09.B.T-03 More Robust Results through Normalisation using the Central Values from the Inventory Database

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Normalisation in LCA allows comparing impacts relative to each other by making results dimensionless, enabling aggregation through weighting and consistency checks against a reference. LCA relies on external normalisation for meaningful references. Previous studies highlighted the importance of aligning normalisation references with inventory data and proposed values linked to LCI databases. This work improves the approach by replacing the geometric mean with the median to enhance robustness, illustrated using the Ecoinvent database and the Environmental Footprint (EF) 3.1 method. LCA impacts and flows are often log-normally distributed, making geometric mean a relevant option. However, some flows are negative, rendering geometric mean calculation impossible and previous estimation algorithms partially unsatisfactory. For log-normal distributions, the geometric mean and median are theoretically equivalent, motivating the use of the median as the reference. Calculations were performed using Ecoinvent 3.11 for APOS, Cut-off, and Consequential system models with Brightway 2.5, and results were compared with EF 3.1 literature values. Strong log-log correlations were observed between geometric mean and median for all models ($r > 0.999$). Comparisons with EF 3.1 show high correlation ($r > 0.98$) but discrepancies regarding certain impacts are being discussed. Using the median of inventory databases as the normalisation value produces more robust and consistent results, minimizing biases. In absolute sustainability assessments, impacts are normalised according to the safe operating space, raising issues of consistency in metrics and system boundaries. Data-consistent normalisation mitigates these issues, while still requiring alignment with planetary boundary considerations, offering a practical alternative to reconciling all units and system differences.

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5.09.B.T-04 Damage-Based Weighting Factor for Human Health-Related Impacts

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Aggregating information to simplify multicriteria decision-making is a common but complex problem. Life Cycle Assessment addresses it in two ways: (1) through modelling up to the damage level and grouping impacts by area of protection, and (2) through less advanced (midpoint) modelling in which aggregation is obtained through weighting.

After normalisation, weighting methods imply choices and face criticism because they depend on value judgments and/or political decisions. For instance, for the Environmental Footprint 3.1 single score, weighting factors are built from a panel approach, meaning that the selection of the panellists can greatly influence the results. However, weighting factors derived directly from damage instead are preferable. Impact categories are then weighted according to their severity. From this, an interesting assumption is that midpoint impacts weighted according to damage should converge towards the contributions of final impacts in the protection areas, and to study possible divergences.

To develop weighting factors based on damage, giving the example of Human-Health-related impacts, epidemiological data from the Lancet Commission on Pollution and Health were used. The most recent updates provide quantified estimates of pollution-related mortality and of climate-change-related mortality. These data were compared, using correlation analysis, with the individualist ReCiPe 2016 endpoint results.

This analysis reveals a significant correlation between the data, showed by the $R=0.84$ and $p\text{-value}<0.05$. Particulate matter shows the highest impact and conversely, Human toxicity cancer and Photochemical ozone show the lowest impact on Human Health damage. The Climate change indicator deviates the most from the

regression line.

These initial results have demonstrated a promising and significant relation between epidemiological data and endpoint results on Human Health damage for the ReCiPe Individualist perspective. The same data collection strategy will be applied for the Ecosystem Quality and Resources areas of protection to complete the weighting factors based on damage.

This exploration could strengthen the robustness of the methodology used and better reflecting weightings associated with actual damage on Earth.

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5.09.P Life Cycle Assessment for Sustainability Decisions: Methodological Challenges and Future Directions

5.09.P-We382 Qualitative And Quantitative Comparison Between Emerging Guidelines for Automotive Life Cycle Assessment in Europe: A Case Study of a Battery Electric Vehicle

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Life cycle assessment (LCA) has become a key tool in the automotive sector for shaping environmental strategies and ensuring policy compliance, highlighting the critical role of methodological decisions. This study analyzes three major recent LCA guidelines developed through prominent European harmonization efforts: TranSensus LCA, Catena-X, and the United Nations Economic Commission for Europe (UNECE) Automotive LCA Guidelines. A qualitative comparison reveals substantial alignment among the guidelines, with notable differences primarily in the modeling of electricity and the treatment of multifunctionality, where methodological flexibility still exists. When applied to a battery electric vehicle, a quantitative comparison reflecting expected practical applications indicates less than a 10% variation in most impact categories. However, certain intra-guideline methodological choices produce larger discrepancies, such as 27% for the Circular Footprint Formula and 11% for static electricity modeling in climate change impacts. Overall, the results suggest that horizontal harmonization across guidelines is largely achieved, whereas vertical harmonization within individual guidelines and their consistent practical implementation remain areas for further development. The findings of this study provide important insights into the current state of harmonization, the consequences of applying different approaches. They also draw attention to the areas where further alignment should be prioritized to make the efforts towards harmonization more effective in terms of fostering consistency and reliability in automotive LCA practice.

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5.09.P-We383 Assessing Individual Carbon Footprints in Higher Education in Taiwan: A Behavior-Linked Emission Model

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Higher education institutions contribute to sustainable development through education, research, and community engagement. This study examined the relationship between carbon emissions and knowledge, attitude, and practice (KAP) regarding carbon emission-related issues among university students. A cross-sectional online survey was conducted in the period September-December 2024 across eight universities in central Taiwan, yielding 293 valid responses. Daily per-capita carbon emissions were estimated based on activity categories and emission factor.

Results showed that daily living accounted for the largest share of emissions (88%), followed by transportation

(20%) and academic activities (10%), with an average daily emission of ~12 kg CO₂e per person. Significant differences in emissions were observed across living arrangements and household sizes ($p < 0.05$). In the KAP analysis, attitude and practice demonstrated a strong positive correlation ($r = 0.59$, $p < 0.01$), while practice and total carbon emissions were negatively correlated ($r = -0.12$, $p < 0.01$).

This study quantifies student-level carbon emissions in Taiwanese higher education and addresses a research gap by linking individual-level KAP variables with carbon emission estimates. The findings provide a basis for understanding the distribution of emissions and for informing sustainability-related policies in higher education institutions. Future initiatives may focus on campus infrastructure and education to support sustainability and net-zero carbon goals.

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5.09.P-We384 Decision Making Towards the Hydrogen Economy: Product Environmental Footprint Category Rules for Fuel Cells for Electricity Production – Proposal and Results of the HyPEF Project
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The energy transition requires low-carbon solutions. Hydrogen systems are high on the political agendas. To prioritise solutions, their environmental performance needs to be evaluated in a comparable, consistent and transparent way. While Life Cycle Assessment (LCA) is a prominent approach for evaluating environmental performance of products, so-called Product Category Rules (PCRs) can be drafted to achieve comparability and consistency. When using the Product Environmental Footprint (PEF) methodology, as outlined in the European Commission Recommendation 2021/2279, such rules are referred to as PEFCRs. In the frame of the European HyPEF project (<http://hypef.eu>), initial versions of PEFCRs have been drafted for three hydrogen-related product categories (i.e. “Devices for hydrogen production”, “Tanks for hydrogen storage”, and “Hydrogen fuel cells intended for electricity production”). They are “shadow PEFCRs” because they have been developed without direct involvement of the European Commission. After having also integrated feedback from an external review panel, they have been applied to fuel cells. This work covers how hydrogen systems are categorized into eleven product types. Furthermore, it explains the specific rules for fuel cells, including how representative products are chosen, how the functional unit and system boundaries are defined, which environmental impacts are most relevant, and which additional technical or environmental information is proposed to be provided. Results are shown for one representative product and three types of fuel cells: low-temperature proton-exchange membrane (PEM) fuel cells, high-temperature PEM fuel cells, and solid oxide fuel cells. Key challenges, such as limited data and defining the functional unit and the representative product, are also discussed.

Disclaimer/Disclosure:

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5.09.P-We385 Integrated Tool for Quantification and Communication of Carbon Footprint in Brazilian Livestock Production

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Population growth and increasing purchasing power, especially in developing countries, are expected to drive a global rise in the demand for animal proteins such as meat and milk by 2050. In this context, the livestock sector faces the challenge of increasing productivity in a sustainable manner, reducing environmental impacts without compromising animal welfare or food security. To address this challenge, Brazil has developed the “Low-Carbon Milk” and “Low-Carbon Beef” programs, which establish guidelines for production systems with lower greenhouse gas (GHG) emissions. As technical support for these initiatives, the Low-Carbon Livestock

Calculator (LCLC) was developed to estimate the carbon footprint of livestock products such as meat and milk produced under tropical conditions. This calculator was built by combining equations proposed by the Intergovernmental Panel on Climate Change (IPCC) with the Life Cycle Assessment (LCA) methodology. Emission factors associated with animal feeding, energy use, and transportation are included as input data in the LCLC, complementing the results generated by the IPCC equations. For beef production systems, the LCLC adopts a cradle-to-slaughterhouse gate system boundary, whereas for milk production systems, it applies a cradle-to-farm gate boundary. The LCLC provides estimates of the carbon footprint of beef and milk, defined as the balance of GHG emissions and removals occurring throughout the product life cycle. The functional unit for beef systems is 1 kg of processed meat, comprising forequarter, hindquarter, and short plate cuts, each representing a reference flow. For milk production systems, the functional unit is 1 kg of fat- and protein-corrected milk (FPCM). The results generated by the LCLC are intended to support decision-making by producers and industries, although they can also be disseminated in an aggregated form for regional or national analyses. These results can also serve as instruments for communication and strategic planning, contributing to the development of mitigation strategies and the enhancement of GHG removals toward more efficient and sustainable livestock systems. Furthermore, the LCLC can provide technical input for the formulation of public policies and certification schemes aimed at promoting and valuing low-carbon livestock products.

5.09.P-We386 Developing a Scalable Carbon Budgeting Framework for Decarbonising UK Highway Maintenance: Evidence from a Cornwall Case Study

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Highway maintenance contributes significantly to the indirect carbon impacts of the transport sector, yet no established method exists for translating national Net Zero targets into operational carbon budgets for routine road maintenance. This study develops a scalable, evidence-based carbon budgeting framework for UK local authorities, demonstrated through a corridor case study in Cornwall.

The work begins by identifying the challenge of aligning local maintenance practices with the UK's legally binding carbon budgets. Through a systematic review of global and sectoral carbon budgeting approaches and decarbonisation pathways, we assess the strengths and limitations of existing methods and their relevance to the material- and energy-intensive nature of highway maintenance.

To address this challenge, we design a mixed-methods framework that integrates baseline life-cycle emissions, sector-specific decarbonisation pathways, scenario modelling, and stakeholder insights. The methodology uses a grandfathering-based budgeting approach with 2022 emissions as the reference year, combined with material- and activity-specific reduction trajectories drawn from national and European guidance. The A30 Hook Corridor test case illustrates the framework using detailed emissions data for asphalt, concrete, plastics, steel and iron, transport, plant operations, waste, chemicals, and ancillary materials.

Results indicate that maintenance-related emissions could fall to roughly 10% of 2022 levels by 2050 when sectoral pathways are applied. Asphalt remains the dominant source of embodied carbon, while transport and plant emissions decline sharply as electrification and hydrogen technologies mature. Scenario modelling reveals substantial variation in reduction potential across activities, underscoring the need for targeted interventions and activity-level planning.

The findings show that corridor-level carbon budgeting offers a practical link between national climate commitments and on-the-ground maintenance planning. The framework enables authorities to allocate, track, and adjust carbon budgets in line with Net Zero objectives and can be scaled across different operational contexts. Future work will expand the framework to support broader adoption and guide long-term decarbonisation strategies in the UK highway maintenance sector.

5.09.P-We387 Ecotoxicity Impacts of Chemical Pesticides Applied to Vegetable Crops in Finland During 2003–2019

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On a global basis information on the use of chemical pesticides on different crops and their related environmental impacts over time is currently lacking (Mark et al., 2024). Mapping the use of pesticides is the

basis for the analysis of ecotoxicological impacts for any crop production system and agricultural area. To address this gap, we conducted a multi-year study to understand dynamics in impacts and possible shifts in main contributing pesticides.

We showed impacts of chemical pesticide use in carrot, potato, swede, and fresh pea production in southwestern Finland over 17 years. The data cover chemical pesticide usage on more than 100 field vegetable farms in southwestern Finland in 2003–2019. We used widely used life cycle impact assessment models to estimate related emissions into the environment and assess potential freshwater ecotoxicity impacts of 57 pesticides applied on the studied crops.

Pesticide usage and related ecotoxicity impacts varied across crops, years and pesticide target classes. The usage and impacts of pesticides used on carrots and fresh peas increased over time. In contrast, usage and impacts decreased over time for swede. For potatoes, the usage of pesticides increased, while the impacts decreased over time, although the differences were not statistically significant. Overall, the amounts of pesticides used were not directly related to their environmental impacts, mainly due to differences in ecotoxicity potency across pesticides. In our study, the impacts were dominated by a limited set of substances. The most hazardous substances were the herbicides acifluorfen and linuron, the fungicide mancozeb and the insecticides lambda-cyhalothrin, cypermethrin, dimethoate and alpha-cypermethrin.

Our results provide extensive information of impacts of pesticides on selected field vegetables in southwestern Finland. We were able to rank chemical pesticides in terms of their impacts under real-world applications, and derived recommendations for replacing the most hazardous chemicals identified with less harmful alternatives. This can inform pesticide substitution and other impact reduction measures in Finland and has the potential to be applied to other pesticides, crops, and regions.

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5.09.P-We388 Normalization And Weighting As Tools For Product Development – A Case Study Of An Electrode

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Individual indicator results are often the go-to when talking about life cycle assessment (LCA) and comparing different options. This poses a problem, when speaking about total environmental impacts as it is not easy to compare specific units and make decisions about the relevance of each result. Normalization and weighting help unify results and together with a single score result they help decision making especially in situations where materials are replaced by new and innovative ones.

This is relevant for printed electronics where materials are evolving at a rapid rate but may struggle with things like higher energy demand and subsequent climate or resource impacts while other environmental impacts may be lower than their traditional counterparts. Normalization and weighting give better insights to technology developers on what should be the focus of research and development actions. It might be that the envisioned change in one material is not that relevant after all when looking beyond a single impact category result, like climate change.

This study is part of the three-year EU SUSTRONICS project which promotes sustainable electronics. The study focuses on electrodes for wearable health monitoring devices. LCA is used to evaluate the sustainability of different material combinations, focusing on the environmental impacts of substrates, conductive inks, and adhesive biomaterials but also including other components and life cycle stages.

The assessment has been carried out throughout the project, for three generations of the product. Results for the final 3rd generation are expected by the end of the project in May 2026. The assessment has progressed from only climate change impacts of substrates and inks in generation 1 to full LCA of the whole life cycle using PEF3.1 method with all its 16 impact categories and recommended normalization and weighting factors.

Electrode life cycle is divided into manufacturing, use and end-of-life stages where manufacturing is further divided between all the materials used to see their individual contributions. Results will be presented as normalized and weighted impacts and comparison will potentially be done using the PEF single score.

Findings of the study are used to guide product development and further understanding whether switching to greener conductive materials and bio-based substrates is worth the potential loss in performance.

5.09.P-We389 Harmonizing Life Cycle Inventories for Agricultural Products: A Swiss Case Study on Environmental Benchmarking and Reporting

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The use of Life Cycle Assessment (LCA) to quantify the environmental impacts of products is central to sustainable decision-making as well as reporting and informing consumers. However, in the agri-food sector, the methodological diversity inherent in LCA—due to varying assumptions, data sources, and regional contexts—presents significant challenges in creating consistent, comparable environmental benchmarks. Benchmarks are hereby understood as average impacts of typical production systems.

This study presents a strategy for generating parameterized Life Cycle Inventories (LCIs) of reference systems from farm-level to product-level, specifically addressing gaps in country- and production-specific environmental impact data. By applying this approach to eight commonly produced and consumed Swiss food products—apples, tomatoes, potatoes, carrots, winter wheat, sunflower oil, milk, and pork—we aim to provide robust environmental benchmarks that can support decision-making in industry, policy and the public.

Key methodological challenges addressed include harmonizing LCIs across agricultural practices and food processing stages thus contributing to a better comparability across LCIs, as well as parametrizing LCIs to increase the flexibility in the calculation of environmental impact results. Results indicate that agricultural production consistently drives environmental impacts across all product categories, although processing stages—such as oil extraction or cereal drying—can amplify impacts. Notably, post-harvest storage can increase global warming potential by up to 35% for each additional two months of storage.

In addition to developing a practical methodology, this research also focuses on effective communication strategies for disseminating these benchmarks to diverse audiences. Environmental impact data will be communicated through consumer-facing platforms, including a web-page and a consumer newspaper, alongside more technical publications targeted at industry and scientific stakeholders.

This research underscores the importance of harmonizing approaches and overcoming methodological challenges to ensure consistent, transparent, and meaningful environmental benchmarks. It therefore contributes to ongoing efforts to standardize LCA practices and improve the reliability of environmental reporting across sectors.

5.09.P-We390 Life Cycle Assessment in Regulatory Science: the should, could and shouldn't

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Life Cycle approaches are used in many aspects of policy and regulation, including emissions reporting, however a full life cycle assessment is often not carried out, or even necessary. In some cases this simplifies methodology and enables faster reporting, however it can also lead to unintended consequences. The UK Life Cycle Assessment Regulatory Science and Innovation Network was set up in 2024 to help support the UK government move towards its net zero goals by providing evidence, and developing robust, harmonised methodologies.

Guidance on where LCA should, could and shouldn't be used in regulatory science have been developed. For example, digital product passports are a positive step forward in the transfer of reliable primary data and should be used to improve product assessments; LCA should be used in the development of biomass policy; results of LCA on low TRL processes should only be presented with caveats so as not to be misleading; individual product LCAs over a wide range of products would be too time consuming.

In addition, working groups have explored the use and availability of data, including where proxy data is not satisfactory, and the harmonisation needed to enable transparent, reliable comparison of products.

Examination of how data issues interact with methodological choices across real regulatory use cases including EPDs, CBAM, ecolabelling, investment decisions, and sector specific rules have been explored, identifying the research and infrastructure priorities needed to strengthen the robustness and consistency of LCA in regulatory and policy contexts.

Evidence generated through the network fed into a UK government consultation on low carbon product emissions reporting.

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5.09.P-We391 Integrating LCA and Energy Modelling in Buildings with Green Roofs: Methodological Challenges

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Buildings are a major contributor to greenhouse gas emissions, mainly due to energy consumption for heating, cooling and electrical devices. In the European Union, approximately 75% of the building stock is energy inefficient, underscoring the urgent need for energy-efficiency measures. This challenge is addressed through directives such as the European Green Deal and the Energy Performance of Buildings Directive, which promote the integration of passive energy-efficiency strategies to enhance building and urban sustainability. Among these strategies, green roofs - vegetated structures above buildings - have emerged as a promising mechanism to improve the building's thermal performance. The integration of LCA and energy modelling provides a robust methodological foundation for evaluating the influence of green roofs as a passive energy-efficiency strategy, which is essential for outlining a framework to incentivise their use and reduce the building's energy consumption and potential environmental impacts. Previous studies have assessed the influence of green roofs on a building's thermal performance, quantifying heating and cooling energy consumption relative to a conventional flat-roofed building, often combining Life Cycle Assessment (LCA) and energy modelling. It has been common to define the functional unit (FU) based on the green roof's area, while accounting for its lifespan. However, the definition of FU remains an ongoing debate among experts, as it currently does not comprehensively capture the integration of green roofs and buildings and their functionality. In this sense, this work proposes expanding the system boundary to include both the green roof and the building, given that the study aims to assess the environmental effects of green roofs on the thermal efficiency of a residential building. The FU could consider the volume of the residential building, specifying the number and typology of floors, the roof area and its lifespan, and the amount of energy required to guarantee a comfortable temperature for the coldest and warmest conditions.

5.09.P-We392 Evaluating Urban Greening as an Urban Heat Island Mitigation Strategy in Tokyo Through Life Cycle Assessment.

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Global temperatures have risen by more than 1.5°C above pre-industrial levels, and cities are experiencing more intensifying heat due to the Urban Heat Island (UHI) effect. In Tokyo, UHI has increased local temperatures by up to 3.2°C, driven by heat-retaining materials, dense built structures, and declining tree canopy coverage.

Urban greening is widely recognized as an effective cooling strategy, as trees provide shade and enhance evapotranspiration. However, urban trees also generate emissions across their life cycle through planting, maintenance, and eventual removal. This raises the question of whether their long-term cooling and CO₂ sequestration benefits outweigh the environmental costs. This study addresses this question using a Life Cycle Assessment (LCA) approach.

The research examines four common Japanese tree species to determine how many trees of each species are required to reduce temperatures within a target Area of Interest (AOI) by 1°C. Because canopy size, growth characteristics, and CO₂ absorption vary by species, understanding these differences is essential for selecting the most efficient cooling strategy.

In addition, to further compare, solar-reflective building coatings, as an alternative UHI mitigation method are also evaluated for their cooling effectiveness and greenhouse gas emissions.

Microclimate simulations are conducted using ENVI-met, while SimaPro is used to quantify life-cycle emissions. Together, these tools allow for a comparison of biological and non-biological strategies in terms of both cooling performance and environmental impact.

The anticipated outcomes will clarify which tree species offer the best balance of cooling and low emissions, and how they compare to reflective surface technologies. The findings aim to support urban planners and policymakers in designing effective, low-carbon strategies for reducing UHI in Tokyo and similar high-density cities.

5.09.P-We393 Scope 3 Environmental Impacts of Workplace IT: Finding the Balance Between Insights and Actions

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Scope 3 emissions, defined as all indirect greenhouse gas (GHG) emissions (excluding Scope 2) that occur in an organisation's upstream and downstream value chain, represent an important part of its total greenhouse gas footprint. Information and communication technology (ICT) is a relevant source of Scope 3 emissions, and the this sector is estimated to contribute 2–4% of global GHG emissions. Workplace ICT can form a relevant share of organisational Scope 3 impacts. In many cases, workplace ICT is not reported in detail in corporate social responsibility (CSR) reporting. Connections between hardware and software, software-as-a-service (SaaS), hardware replacement policies, and user behaviour are not always tracked consistently, and the translation of CSR goals into concrete ICT interventions is unclear. Life cycle inventories (LCIs) for hardware are also limited. This makes it difficult for organizations to gather reliable Scope 3 data and identify improvement options.

This study presents a Scope 3 accounting framework for workplace ICT to support CSR reporting and guide decisions. Workplace ICT includes end-user devices, workplace software, and the associated cloud-based services that support routine organisational activities. The analysis does not cover the deployment of ICT solutions such as back-end business applications or large-scale data-processing systems that are not directly linked to these workplace services. The framework is demonstrated with real-life organisational use cases, using primary asset and usage data from Dutch organizations. We combine these data with background inventories and apply a consequential life cycle assessment (LCA) to estimate the Scope 3 impacts of workplace ICT. Then, we focus on mitigation interventions along two main organisational levers: procurement decisions and employee behaviour.

The study evaluates the mitigation potential of selected interventions. For procurement, this includes extending device replacement cycles and choosing lower-impact devices or cloud options. For employee behaviour, we consider interventions like reducing video-conferencing resolution. An effect–effort matrix, informed by ICT procurement staff, prioritizes interventions by environmental benefit and feasibility. Organisations can use the framework to develop tailored workplace ICT impact profiles and prioritise interventions. Overall, our contribution is to link Scope 3 impact insights for workplace ICT with concrete mitigation actions.

5.09.P-We394 On The Relevance of Lab-scale Life Cycle Assessments

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In recent years, life cycle assessment (LCA) has been increasingly applied to emerging technologies that currently exist only at the laboratory scale. These lab-scale LCAs are typically conducted using the available experimental data from early-stage processes without considering technological upscaling or maturity. Consequently, such studies often raise concerns regarding their representativeness and relevance, particularly the extent to which the environmental hotspots identified at laboratory scale correspond to those observed at industrial scale.

This study reviewed and analyzed over 30 published LCAs that reported both laboratory- and industrial-scale results across diverse technological domains, including chemical synthesis, wastewater treatment, food processing, carbon capture, and emerging batteries, to systematically evaluate how absolute environmental impacts and hotspots change with scale. The results show that absolute environmental impacts typically decrease by up to 99% during upscaling, primarily due to improvements in energy and material efficiency, process integration, and waste reduction. The findings also indicate that lab-scale LCAs often capture environmental hotspots that may diminish or disappear when the system is scaled up to industrial production. The study further discusses which types of research questions lab-scale LCAs can appropriately address and for which stakeholder groups they offer meaningful insights. Additionally, it highlights that incorporating sensitivity analyses or discussing how environmental hotspots may change with upscaling can make lab-scale LCA results more relevant and informative for a broader audience.

5.09.P-We395 Evaluation of Net-Zero Strategies for Construction Products Using Life Cycle Assessment
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The concept of net-zero has gained a lot of attention following the Paris Agreement and EU regulations, yet its definition and calculation methodology have been interpreted in many ways. In the building sector, terms such as Net-Zero Energy Buildings focus on minimizing operational energy rather than the impact of construction materials. Recently, the Net-Zero Carbon Buildings Standard has emerged, addressing both operational and embodied emissions. The net-zero concept is also widely used in business. Many companies have pledged to achieve net-zero by 2050, but these commitments often lack clarity on whether they rely on major emission reductions or offsets. To address this, the Science-Based Targets initiative (SBTi) introduced the Corporate Net-Zero Standard, which requires companies to reduce at least 90% of emissions before offsetting the rest. Beyond buildings and business, it remains unclear how the concept of net-zero can be generally applied at the product level.

This study investigates whether specific products can be considered to have net-zero emissions in a life cycle perspective, taking construction products as a case study. The methodology combines a literature review of strategies across main construction products, with Life Cycle Assessment (LCA) modelling. Strategies are considered at two levels: material and product, which can demonstrate that achieving net-zero emissions requires different types of strategies- on the production and on the design side. To illustrate the approach, a window is used as an example. At the material level, general strategies identified for glass include waste heat recovery, carbon capture and storage, use of fossil-free energy, and increased recycled content, together with specific innovations like hybrid melting furnaces. At the product level (window in this context), strategies include substituting aluminium frames with wood-aluminium composites, integrating solar control films, and replacing metallic spacers with warm-edge alternatives.

The expected outcome is to quantify the effectiveness of each of the strategies and to evaluate whether their combination can achieve the net-zero emission material or product. While the window serves as an example, the approach is expected to be applied to several construction materials and products. This work will contribute to clarifying the concept of net-zero in products and provide insight into reducing the environmental impacts of materials in the construction sector.

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5.09.P-We396 Grounding future regulations into common standard for pharmaceuticals LCA: hands-on comparison of UK standard and French guideline

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Regulatory developments across Europe are fundamentally reshaping the life cycle assessment (LCA) landscape for pharmaceutical products – transforming it from a voluntary exercise to a prerequisite for market access. As these direct regulations come into force, the ability to deliver robust and consistent LCA results is becoming essential for pharmaceutical companies seeking to comply with evolving legislative requirements and remain competitive in the European market. The only standard dedicated to the LCA of pharmaceuticals – PAS 2090 – has been published in December 2025 by the British Standards Institution (BSI).

Meanwhile, the French government has already introduced a methodological guidance for pharmaceutical products LCA with a specific focus on Product Carbon Footprint (PCF). This guidance does not fully align with the PAS 2090 standard.

Although it is a well-intentioned effort to support the industry in disclosing PCFs, this development highlights the risk of regulatory fragmentation and the importance of harmonizing standards at the European level, and eventually globally, to ensure consistent, efficient and comparable environmental assessments within the pharmaceutical sector. In this study, we aim to provide a comparative analysis of the PAS 2090 standard and the French PCF methodological guideline. We identify key differences with respect to methodological approaches and data requirements, which strongly influence the final impact assessment results. We focus on climate change impact category, as this is the only one in scope of the French PCF methodology. The comparison is based on practical case study applications on synthetically derived medicines and biologics. The findings of this study underscore the importance of adopting a common, industry-specific standard for pharmaceuticals' LCA when implementing new country-specific regulations. Divergent methodologies risk generating non-comparable results across countries and may significantly increase both the costs and complexity associated with conducting LCAs for medicines. Furthermore, such fragmentation presents substantial challenges for future harmonization

of results at the European level, ultimately hindering regulatory alignment and the effective assessment of environmental impacts within the pharmaceutical sector.

5.09.P-We397 Cradle-to-grave LCA of Ethinyl Estradiol: Production hotspots and End-of-Life Burdens

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While pharmaceuticals are introduced to improve quality of life, production and disposal cause environmental impacts leading to loss of life years and loss of biodiversity. Life cycle assessment (LCA) can guide sustainability decisions, but it remains constrained by scarce primary data for active pharmaceutical ingredient (API) synthesis, and limited integration of end-of-life impacts. A cradle-to-grave LCA of ethinyl estradiol is conducted, a widely used synthetic estrogen with documented aquatic ecotoxic effects at low concentrations. The objective of this study is to quantify environmental hotspots across multiple impact categories from ethinyl estradiol synthesis at an EU-based API manufacturer to final disposal, and to assess improvement options through sensitivity analyses. The functional unit is 1 kg of ethinyl estradiol and the system includes synthesis, formulation, packaging, distribution, patient use, wastewater treatment, and environmental fate. Industrial primary data for ethinyl estradiol synthesis are combined with background data from ecoinvent. Upstream chemicals missing in databases are modelled using an upscaling-based estimation method. For the use and end-of-life stages, substance-specific pharmacokinetics are combined with the ePiE model (wastewater removal and fate estimation) and linked to freshwater ecotoxicity. ReCiPe 2016 was used for the impact assessment with a systematic stepwise procedure. Preliminary contribution analysis indicates that upstream starting-material production contributes more than 40% across selected midpoint categories (e.g., climate change, human toxicity, and ecotoxicity), whereas ethinyl estradiol synthesis at the European plant contributes less than 29%. A solvent reuse scenario reduces selected midpoint indicators by 19–46% relative to the base scenario reflecting current practice. This study is the first to present a cradle-to-grave LCA of ethinyl estradiol that combines industrial primary data for API synthesis with explicit modelling of end-of-life ecotoxicity. The framework of this study can be used consistently and transparently to compare a range of impact categories to identify improvement options from a life cycle perspective. The study provides a transparent basis for decision makers (e.g., manufacturers and regulators) to prioritise improvement action and implement policy measures such as stakeholder responsibilities for waste treatment within the value chain.

5.09.P-We398 Miscanthus to Xylo-oligosaccharides and Biocarbon: Innovating Feed, Food and Steel Industries with the MAXFEED System

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The perennial grass *Miscanthus* holds substantial promise as a lignocellulosic feedstock for a biorefinery approach that delivers both climate mitigation and high-value bioproducts. Xylo-oligosaccharides (XOS), for instance, are valuable prebiotic ingredients used in food, feed, and health applications. XOS in an animal's diet can reduce CH₄ emissions for cattle, specifically. Alongside, Biochar offers a renewable and lower-carbon alternative to replace part of metallurgical coal in blast furnaces, enhancing sustainability. To highlight the environmental opportunities, challenges, and uncertainties of producing biochar and xylo-oligosaccharides (XOS) with *Miscanthus*, a life cycle assessment (LCA) was conducted. The impact of XOS as an animal feed supplement was forecasted considering raw beef production in the UK's market³ since the prebiotic effect of XOS showed potential to reduce CH₄ emissions^[2], while biochar was used as an alternative feedstock to coal in the steel-making industry. The MAXFEED system operated alone and combined with the food and steel systems. The system boundary was cradle-to-gate, including the following systems: *Miscanthus*'s farm, MAXFEED factory (biochar and XOS), steel-making (pig iron production), and cattle's farm (raw meat). For 1 kg of products as functional unit (pig iron and meat (97.2%)), significant reduction of global warming (GWP100a) and land use (LUP) potential was observed. Negative GWP100a during the biomass production mitigated environmental burdens allocated to products in comparison to their benchmarks - pig iron, beef, and coal. A significant step towards carbon-neutral bioproducts due to a 89% reduction in GWP100a was observed when compared to the conventional pig iron production—the carbon footprint falls to 0.20 kg CO₂ eq. per kg of pig iron produced with biochar, mitigating approximately 50% of the steel footprint. Alongside, the combined benefits of carbon sequestration during biomass production and the reduction in CH₄ emissions resulting from the prebiotic property of XOS can reduce the overall LUP of meat production from cattle fattened with XOS supplementation. LUP decreased by about 93%, with the index dropping to ~489 [dimensionless] per kg of meat produced, compared to animal feed production without XOS supplementation. Scalability challenges for the UK's landscape were identified, suggesting opportunities to explore different markets and applications for the bio-products.

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5.09.P-We399 A Call for Revising ISO 14040 and 14044

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Standards within ISO are subject to systematic review every five years. In these reviews, a decision is taken whether to revise the standards. Since most of the text in the generic standards for life cycle assessment (LCA; ISO 14040 and 14044) will be nearly 30 years old at the upcoming review, the arguments for revising the standards are strong. Clarification of the existing terminology in the standards is needed, because other standards and guidelines that are based on ISO 14040+44 diverge in the interpretation of terms such as “system expansion” and “underlying physical relationships”. Even the basic terms “life cycle” and “product system” need clearer definitions. The current standards are not sufficient to know if the life cycle is a system of physical flows or a sociotechnical system – or even if it is the system modelled in an attributional LCA or in a consequential LCA.

Since the methodology has developed, we would also benefit from clear definitions of new terms such as attributional, consequential, prospective, and dynamic LCA, foreground and background systems, primary and secondary data, etc.

The understanding of how the appropriate methods depend on the context of the study have developed. Revised LCA standards can account for this through explicit flexibility in methods or through a structure of methods designed for different contexts.

Updates in the standard texts are needed because LCA practice has developed. As an example, the current requirement to qualitatively and quantitatively describe all unit processes is outdated, because commercial databases have changed the conditions for data collection and reporting.

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5.09.P-We400 Enhancing Supply Chain Transparency in Electric Arc Furnace Stainless Steel Production: A Decision Support Tool Based on Life Cycle Assessment

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Stainless steel production via Electric Arc Furnace (EAF) is characterized by a high share of indirect environmental impacts due to the intensive use of alloying elements. Despite this, the contribution of raw materials procurement to the overall impact of different stainless-steel grades remains insufficiently quantified, limiting the accuracy of corporate sustainability reporting, the identification of effective mitigation strategies and transparent communication to stakeholders.

This study develops a cradle-to-gate Life Cycle Assessment (LCA) model that integrates direct process emissions with upstream impacts associated with raw materials, energy supply, transport and auxiliary operations. The model covers a broad portfolio of stainless-steel grades and enables a grade-specific environmental characterization. Primary data on raw material inputs, electricity consumption, process emissions, waste and water flows were collected at facility level, while upstream processes were modelled using ecoinvent 3.11. LCA calculations were performed with Brightway 2.5 and impact assessment was performed with the Environmental Footprint 3.1 method, including normalization and weighting to derive single-score indicators.

Results demonstrate that raw materials systematically account for more than 90% of total impacts across all stainless-steel categories. Duplex and austenitic grades show the highest single scores, followed by precipitation-hardening, ferritic and martensitic steels. The dominant contributions originate from nickel, chromium and molybdenum related raw materials, mainly ferroalloys, whereas the influence of direct emissions and in-house energy use is comparatively limited.

By providing steel grade-level granularity, this work offers an effective approach for corporate reporting and stakeholder communication. The developed model serves as a dynamic decision-support tool, enabling steelmakers to optimize strategic sourcing, for instance, by mitigating financial risks associated with the EU Carbon Border Adjustment Mechanism (CBAM) on extra-EU ferroalloy imports. Furthermore, it allows customers to accurately calculate their indirect (Scope 3) emissions based on the specific steel grade purchased, ensuring compliance with current regulations. Finally for policymakers, these findings offer the evidence needed to refine steel production environmental benchmarks, ensuring that regulations include data about the most critical production mix in the industry.

5.09.P-We401 Environmental sustainability benchmarking in the construction sector

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An important contribution to drive an environmentally sustainable construction sector is the definition of benchmarks for environmental impacts, enabling measurement and comparison of environmental performance against established standards and targets. The approach has reached increasing attention over the past years. However, there is still no consensus on sector- or product-specific benchmark values, nor on the methodologies for calculating them. This study aims to evaluate existing environmental sustainability benchmarking approaches for the construction industry.

For that, the PRISMA approach was applied for three different types of sustainability benchmarking in construction: 1) limit values, i.e. the upper or lower acceptable performance level; (2) reference values, representing state of the art or best practice performance; and (3) target values, defining desired performance to be achieved. The databases Scopus and Web of Science were used for the time period until 2025 (August). Studies were included in the systematic literature review if they developed either a benchmarking method or benchmark values in construction. They were analysed with regard to their underlying data foundation, methodologies, and existing benchmark values for construction products.

To evaluate existing values, GWP benchmarks for the life cycle stages A1-A3 of the product category buildings were aligned to the same functional unit and then mapped to determine benchmark ranges. It became evident that while environmental sustainability benchmarks appear to have great potential to drive sustainable development in construction, differences in methodological choices result in large variations, questioning their reliability and effectiveness. Main challenges could be identified in heterogeneity in scope, unit, terminology, and allocation rules, as well as a lack of comprehensive data foundations. An overview will be provided of current benchmarking practices along with their associated opportunities and challenges. These insights into benchmarking methodologies do not only benefit stakeholders within the construction sector but will also offer valuable perspectives applicable across various fields of study concerning effective integration of sustainability assessment results into policy- and decision making.

Disclaimer/Disclosure:

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5.09.P-We402 Beyond the Cell: Unlocking Sustainability in Battery Pack Design for Electric Mobility

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The transition to electrified mobility and renewable energy storage has increased the demand for sustainable battery technologies. While most research focuses on cell-level solutions, pack-level components—representing 30–40% of system weight and involving complex manufacturing—remain underexplored despite their significant environmental, economic, and social impacts. This study addresses this gap by performing a life cycle sustainability assessment of a battery pack designed for semi-solid-state cells, focusing exclusively on pack-level components and processes to ensure applicability across different chemistries.

Using a cradle-to-gate approach, environmental impacts were evaluated with ReCiPe 2016 Midpoint, economic performance through cost modeling, and social risks via the Social Hotspots Database. Results reveal three critical hotspots: the mechanical structure, which drives 56% of environmental impacts due to aluminum-intensive designs; the Battery Management System (BMS), responsible for 57% of costs and major social risks linked to electronics supply chains; and thermal management, contributing 9% of environmental burden but

influencing safety and lifetime.

Mitigation strategies include modularity and recyclability for structural components, design-for-recycling for BMS electronics, and advanced thermal solutions such as phase-change materials. These approaches can reduce emissions, extend lifetime, and support circular economy principles. Findings provide actionable guidance for eco-design, enabling stakeholders to anticipate trade-offs and prioritize interventions early in development, advancing sustainability in next-generation battery systems.

Disclaimer/Disclosure:

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5.09.P-We403 Smart design for a dual-purpose farm level calculation tool for beef cattle – reconciling advising and reporting

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What's the dual purpose?

The beef cattle sector is facing a substantial emission reduction target by 2030. Production conditions for own feed supplies are becoming more difficult and the context less predictable, so farmers need targeted advice. At the same time, individual farmers are increasingly being asked by their customers for climate figures in connection with their reporting obligations (such as CSRD).

What's the challenge?

Farmers often must fill in multiple tools: tailored advice requires detailed and farm specific data, while reporting agencies prefer simplified models with minimal data demand. In both cases, the administrative burden is borne by the farmer. Upfront smart design of the calculation tool can reconcile both purposes, is the lesson learnt in Klimrek dairy.

How does smart design work?

The basis for the calculation tool is the same but the parametrization and added features are tailored to the specific needs. The two goals place different demands on the scan, e.g. completeness, suitability for scenario analyses, time spent, data quality assurance, etc. Two distinct calculation models are built on the same underlying architecture and can be converted into one another depending on the goal. In order to be successful, we use co-creation for both calculation models, each with their unique set of stakeholders.

Expected results

The result will be a practically oriented scan for reporting and a premium scan for advice. Both scans will give insights in the environmental performance of the sector but only the premium scan will allow for in depth data analysis in support of policies.

Why it is relevant?

The concept of a dual-purpose convertible calculation tool is a big step forward in supporting farmers, who are confronted with the obligation to report their footprint and who want to benefit from the insights provided by environmental footprinting for their business, without double work.

Disclaimer/Disclosure:

The tool will be developed starting in Jan 2026 with the financial support of VLAIO (90%) and an additional 10% funding from the dairy beef sector.

5.09.P-We404 Review of using Multi-Criteria Decision Analysis in Life Cycle Sustainability Assessment studies

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Use of Multi-Criteria Decision Analysis (MCDA) methods becomes more and more prominent in Life Cycle Sustainability Assessment (LCSA) studies. From the field of MCDA many different methods available for weighting and aggregating impacts. However, there is little exchange between LCSA practitioners and MCDA specialists. Previous publications show that sustainability assessment practitioners make accidental mistakes when applying MCDA. Thus we took a closer look at the use of MCDA in LCSA studies specifically and want to identify improvement potential for LCSA practitioners.

We performed a literature search in Scopus for the terms 'Life Cycle Sustainability Assessment' and 'Multi-Criteria Decision Analysis' in Title, Abstract and Keywords. The search was performed in February 2025 and we did not regalement the time period. The first article was published in 2011 that fit our search terms. In total we found 120 articles, from which 55 were eligible for the assessment.

The bibliometric analysis showed that on average there is an increasing number of publications over the years. The case studies discuss typical topics in LCA with focus on energy and mobility technologies as well as the construction and the food sector. The publications were published in classic LCA journals, e.g. Journal of Cleaner Production, and topic is pursued by authors all over the world.

We were looking for specific aspects that are crucial for the application of MCDA:

Who is the decision maker for the case study, what type of typology is applied for the decision making problem, what methods are used for weighting and aggregating are used, and who was the topic of uncertainty assessment addressed in the different studies.

We found that MCDA is mostly performed by LCSA practitioners who learned about MCDA while they are doing it. That leads to several problems with the studies from an MCDA perspective. On the one hand, there is most often no clear definition of the problem statement. And on the other hand, there is a mismatch of chosen aggregation methods with applied indicators and weighting methods. Thus, it is of utmost importance that LCSA practitioners who want to perform MCDA become better educated. In contrast, it also means that MCDA experts need to communicate better when certain approaches are valid. A first step would be better aid for selecting MCDA methods.

Disclaimer/Disclosure:

This topic is based on a book chapter that should be published next year in the LCSA Contribution of the Springer LCA Compendium.

5.10.A Life Cycle Impact Assessment Modelling and Application

5.10.A.T-01 GLAM 1.1 - The full midpoint-endpoint Global Life Cycle Impact Assessment Method

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The Life Cycle Initiative, hosted at UNEP, has developed a Global LCIA Method (GLAM) through a science-based consensus process. GLAM 1.0 focused on endpoint/damage level, but missed multiple midpoint indicators. This work aims to presents GLAM 1.1 as a full consistent midpoint-endpoint LCIA method. GLAM 1.1 covers classification, midpoint & damage characterisation, normalisation and weighting. It assesses the life cycle impacts of products on human health (HH), ecosystem quality (EQ) and socio-economic assets (SEA). Three principles were applied to derive the the GLAM midpoint indicators: 1) Use GLAM already defined and commonly used midpoints, selected at the earliest "point of convergence", 2) differentiate between types of effect for HH (e.g. cancer, cardiovascular disease), and 3) Otherwise, divide endpoint damages by the damage of a reference substance.

GLAM provides 1.1 million characterization factors covering 14000 category-flows combinations for more than 17 impact categories. Novelties include the inclusion of the use phase HH impacts of chemicals in products, nutrition & physical activity as well as impacts on workers. EQ impacts considers new factors for marine microplastics, land use fragmentation, terrestrial ecotoxicity and accounts for species richness and vulnerability through spatialized Global Extinction Probability (GEP). For SEA, land use impacts on 5 ecosystem services are considered and a dissipation-based framework is set up for mineral resources.

These damage scores can then be normalized by the average total impact per person worldwide for the corresponding AoP. Normalized global damages are presented for HH, EQ and SEA, showing in particular substantial impacts for climate change, PMs, nutrition and physical activity benefits and dominant impacts of land use on terrestrial ecosystems, of ecotoxicity on freshwater ecosystems and of eutrophication and marine plastics on the marine environment. We will finally present consistent sets of impact category specific midpoint weights and AoP specific weighting factors to respectively apply to normalized midpoint or endpoint damages.

GLAM has benefited from the collaboration of more than 200 scientists from across the world and constitutes a quantified "one Earth" dataset for impacts on both ecosystem and human health. Once implemented in LCA software it will offer valid CFs worldwide, for application to global supply chains, and provide increased ability to avoid burden shifting.

Disclaimer/Disclosure:

GLAM development was supported by the Life Cycle Initiative in particular thanks to the continuous financial support of the Swiss government. A major part of the work was also provided via free work contribution of the more than 200 scientists worldwide supported by their own institution and projects.

5.10.A.T-02 The Environmental Footprint Life Cycle Impact Assessment method: updates for the EF 4.0 version

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The Environmental Footprint (EF) methods, developed by the European Commission, offer standardized frameworks for assessing environmental impacts of products and organizations across their life cycles. Introduced in 2013, these methods (including Product Environmental Footprint (PEF) and Organization Environmental Footprint (OEF)) support policy-making, corporate accountability, and sustainable consumption. The Joint Research Centre (JRC) periodically updates the EF Life Cycle Impact Assessment (LCIA) method to incorporate advancements in impact assessment modeling. The upcoming EF4 LCIA (expected Q2 2026) aims to address two new environmental aspects: biodiversity loss due to land use (using the BIOMAPS model) and environmental impacts due to microplastics emissions (based on MarILCA). Existing categories are revised to reflect improved models, updated characterization factors, including subregional impacts (e.g., water use). Normalization and weighting factors are adjusted to align with new data and address limitations. The EF4 LCIA will expand to 18 categories, enabling more accurate trade-off analyses and comparative assessments. This contribution presents the EF4 methodology, compares it quantitatively to the EF3.1 version, and outlines limitations to guide future research and long-term developments in EF-based sustainability assessments.

5.10.A.T-03 Bringing Pathogens into Life Cycle Assessment: A Spatially Explicit Framework for Health Impact Assessment

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Pathogens originating from wastewater remain a relevant human health concern, yet their impacts cannot be assessed within life cycle assessment (LCA). This gap limits the ability of LCA to evaluate wastewater management, even though treated effluents introduce microbial contamination into surface waters, and overflow events or reduced treatment can amplify pathogen loads. Quantitative microbial risk assessment (QMRA) provides estimates of infection risks, with recreational water use identified as a major exposure pathway. However, QMRA does not capture system-wide trade-offs or allow comparison of wastewater technologies. Bridging QMRA with LCA enables quantification of pathogen-related health impacts from a life cycle perspective.

We present a spatially explicit framework that quantifies human health risks in four steps: fate and transport modelling, exposure assessment, dose-response modelling, and conversion of illness cases to disability-adjusted life years (DALYs). Hydrological and wastewater infrastructure datasets are combined with performance data from Swiss treatment plants to predict pathogen concentrations at recreational sites. Age-specific exposure

doses are derived from population density and behavioral data, and pathogen-specific dose–response relationships estimate illness risks and DALYs. The approach is demonstrated for Switzerland over one year and six pathogens. Concentrations are modelled at designated recreational sites where exposure through swimming is most relevant. We evaluate a baseline scenario reflecting current treatment performance and a degraded scenario with tenfold higher effluent concentrations representing overflows or reduced removal efficiency. Under current conditions, median *Escherichia coli* concentrations at recreational sites are 124 colony-forming unit (CFU)/L in rivers and 54 CFU/L in lakes, illustrating low pathogen levels. When integrating all six pathogens, national health impacts amount to 7 DALYs/year. In the overflow scenario, median concentrations increase (1227 CFU/L in rivers; 540 CFU/L in lakes), and combined pathogen-related impacts rise to 70 DALYs/year. The results show the low current burden and the high sensitivity of pathogen-related health impacts to effluent quality. The framework produces pathogen-specific characterization factors, offers a transparent, scalable approach for integrating pathogen risks into LCA, and supports more comprehensive evaluation of wastewater management options.

5.10.A.T-04 Assessing Global Biodiversity Impacts from Agricultural Intensification (2000–2019)

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Agricultural intensification has raised crop yields but puts increasing pressure on global biodiversity. Despite advances in mapping land-use change and biodiversity indicators, the combined effects of temporally and spatially explicit intensification and crop-specific pressures remain poorly quantified.

This study integrates 20 years of high-resolution land-use intensity data across cropland, plantations, and pastures to quantify biodiversity impacts and attribute them to intensity levels, land-use types, and 143 crop types. We examine how intensification has affected biodiversity since 2000, identifying the land-use types, intensities, regions and crops driving these trends. Compiling a global dataset with three intensity levels (high, medium, low) for crops, pasture, and plantations at 0.25° resolution from 2000 to 2019, we calculated potential species loss (PSL_{glo}) using global characterization factors that consider different intensification levels and linked impacts to the spatio-temporal distribution of crops.

From 2000 to 2019, global biodiversity impacts from agriculture increased by 1.3% PSL_{glo}, but 83% of the increase were concentrated on just 10% of the global agricultural land. Hotspots primarily occurred in the tropics, where high ecological sensitivity coincided with rapid agricultural changes. Increases from high-intensity land use in Southeast Asia, East Africa, and Brazil matched major oil palm, maize, and rice production. Pasture impacts declined globally due to abandonment, though local hotspots persisted. Decreases in low-intensity agriculture in Australia reflected rangeland contraction, while low-intensity expansion appeared in western Brazil and central Africa. Declining impacts in Ecuador, Peru, and Colombia suggest effective conservation measures.

At the crop level, oil palm, maize, and rice accounted for ~50% of the increases in cropland and plantation impacts. Oil palm impacts were concentrated in Indonesia, maize impacts were widespread, and rice impacts grew most in Sub-Saharan Africa. Medium-intensity banana plantations in the DRC made a notable contribution to global impact growth, illustrating how crop type, management intensity, and location interact.

Mitigating biodiversity loss requires managing both expansion and intensification. Crop-specific sustainability standards, improved monitoring of inputs, and integrating restoration into land-use planning are crucial for balancing production with biodiversity conservation.

5.10.B Life Cycle Impact Assessment Modelling and Application

5.10.B.T-01 Characterizing Chemical Toxicity for Life Cycle Assessment Using Machine Learning Models Based on Environmental Footprint – A textile case study

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The transition toward safer and more sustainable chemicals requires reliable tools to identify toxicity at early design stages. However, Life Cycle Assessment (LCA) is often limited by missing toxicity characterization factors (CFs), particularly for novel or emerging substances such as alternative surfactants and bio-based solvents. This study, that was conducted as part of two EU-funded projects (CALIMERO and DESIDERATA), addresses this challenge by developing a machine learning (ML) workflow that predicts both human and ecotoxicity CFs directly from molecular structures, enabling more comprehensive environmental assessments. The framework integrates molecular descriptor generation, feature curation, Gaussian Mixture Model (GMM)-based clustering, and three ML algorithms—Extreme Gradient Boosting, Gaussian Process regression, and Deep

Neural Networks—to model CFs across 16 environmental compartments. Model performance was assessed using data from the Environmental Footprint v3.0 database, achieving coefficients of determination (R^2) up to 0.65 for ecotoxicity and 0.61 for human toxicity. The approach was demonstrated through a textile case study comparing denim washing processes using pumice and synthetic stones. Incorporating predicted CFs revealed that missing toxicity data can substantially underestimate human toxicity impacts by 4 orders of magnitude, altering the perceived environmental ranking of alternatives.

Ongoing work focuses on strengthening prediction reliability to support high-technology-readiness-level applications within the DESIDERATA project. Planned enhancements include integration of Graph Neural Networks, improved applicability domain assessment, and expanded chemical coverage to ensure robust, transparent, and scientifically sound predictions. This work demonstrates the potential of ML to fill critical toxicity data gaps, supporting safer chemical design and more reliable sustainability assessments across industries.

Disclaimer/Disclosure:

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5.10.B.T-02 Effect Factors for Terrestrial Plastic Pollution for Application in Life Cycle Assessment

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Soils are known to be plastic pollution sinks, receiving up to 4-23 times the amount of microplastics discharged into the ocean per year. Although the uptake of plastics by plants and soil biota has been demonstrated and resulting effects have been summarized, this information is currently not usable for life cycle assessment (LCA), a common tool to assess potential environmental impacts of products and processes. The aim of our research is to develop effect factors (EFs) for the uptake of plastics by terrestrial organisms that can be integrated into LCA, and to determine influencing factors.

We derived EFs from species sensitivity distribution (SSD) curves based on effect concentrations obtained from existing experiments. We extrapolated acute values and values that correspond to different dose descriptors by using uncertainty factors and applied statistical testing to determine influencing factors. The SSD curves are based on 3-7 animal species and 14-15 plant species, depending on the inclusion criteria for existing literature. The resulting EFs vary between 0.23 – 0.31 PAF.m³.kg⁻¹, which is in the range of previously published EFs for the terrestrial environment but significantly lower than for the aquatic environment and sediments.

The extrapolation from acute to chronic effect concentrations contributes less to the overall uncertainty factor than the extrapolation from other dose descriptors. The inclusion of values from experiments that measured no statistically significant effect even at the highest tested concentration increases the uncertainty of the data but also the number of species that meet the threshold of $n \geq 5$ for a species to be included in the SSD curve. The Kruskal–Wallis ANOVA and subsequent Dunn’s post-hoc tests indicated no significant overall effect ($p > 0.05$) of the size and shape of the plastics nor of the soil type used for the experiments on the effect concentrations of the three species with the highest number of data points: *E. fetida* ($n=29$), *Z. mays* ($n=28$), and *L. sativa* ($n=23$). This finding is in line with previously developed EFs for other environmental compartments and reduces the complexity of characterization factors for plastic emissions.

Our EFs increase the robustness of previous research regarding the terrestrial environment and allow for an integration with existing fate factors to form characterization factors for plastic emissions for all relevant environmental compartments.

5.10.B.T-03 Development of LCA Characterization Factors for TRWP Physical Effects and Leachate Ecotoxicity

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Tire and Road Wear Particles (TRWP), composed of weathered rubber polymers, chemical additives, and road minerals, are an important source of microplastics in the environment. Detected in all environmental compartments, multiple adverse effects on the biota were reported in the literature, both from the particles themselves and from leached additives. Current LCIA methods for microplastics address only the physical particle effects and do not reflect the combined chemical and physical impact pathways of TRWP, creating a

methodological gap in the impact assessment of these pollutants.

This study develops TRWP-specific characterization factors (CFs) that differentiate and integrate physical and chemical toxicity pathways. A multimedia fate model adapted from SimpleBox4Plastics is used to derive fate factors (FFs), considering TRWP transport, settling, removal, and degradation processes. The model enables the assessment of the redistribution of TRWPs initially emitted into air or onto road surface, towards roadside soil, natural soil, agricultural soil, marine and freshwater, and associated sediments.

To characterize the chemical toxicity of TRWP leachates, species sensitivity distributions (SSDs) were developed and compared to SSDs of key additives frequently found in the leachates, namely 6PPD, 6PPD-q, DPG, Zn. Because TRWP leachate EC10 values express toxicity per mg TRWP/L, the expected aqueous concentration of each additive in a 100 mg TRWP/L leachate was computed, and the associated potentially affected fraction (PAF) was obtained from additive SSDs. The summed additive PAFs were consistently lower than the PAF of the TRWP leachate mixture, suggesting that other additives not accounted for (e.g., MBT, BT derivatives, PAHs) and mixture interactions may also contribute to ecotoxicity.

The resulting freshwater EEF for TRWP leachates is 1985.5 (89.8–43904.4) PAF·m³/kg, about twice as high as available EEFs for the physical effects of generic microplastics. When combined with the FFs determined for each environmental compartment, physical and chemical CFs are formulated to characterize both impact pathways, with preliminary values at 1.52E+05 PAF m³·d/kg of TRWP emitted in freshwater. Additional work is needed to expand terrestrial datasets and harmonize leaching protocols to refine the ecotoxicity CFs and reduce uncertainty.

5.10.B.T-04 Microplastics, Tire Particles, and Cellulosic Fibers in LCA: MarILCA Regionalized Multimedia Characterization Factors

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It is estimated that worldwide, 10 to 40 million tons of microplastics (MPs) are emitted annually into the environment. Once released, MPs redistribute across environmental compartments, affecting organisms in each of them. However, current Life Cycle Impact Assessment (LCIA) methods only partially account for their impacts on ecosystem quality (EQ) by considering a limited number of compartments and particles. This study advances the work of the MarILCA group by integrating the physical effects of MPs, tire and road wear particles (TRWPs), and cellulosic fibers across all environmental compartments into life cycle assessment (LCA).

Characterization factors (CFs) are developed for 20 polymers, 6 cellulosic materials, and TRWPs, for multiple shapes and sizes commonly emitted over the lifecycle of products and services. To this end, particle fate is modelled using the new SimpleBox object-oriented (SBoo) fate model for microparticles, which computes fate factors (FFs) in air; terrestrial (roadside, agricultural, and natural); freshwater (water and sediments); and marine (water and sediments) environments at continental and global scales, following USEtox regionalization across eight regions. SBoo was improved to account for (i) size and shape effects, (ii) degradation using the PlasticFADE model, and (iii) regional marine currents and biofouling through the use of the TrackMPD ocean circulation model.

FFs are combined with exposure and effect factors (EEF), based on hazardous concentration for 20% of species (HC20) in water, sediments, and terrestrial environments separately, to obtain updated CFs and their uncertainties. Impacts across compartments are aggregated using the volume and number of species living in each compartment. For IMPACT World+ (IW+) and ReCiPe, CFs representing regional species loss are derived, while for GLAM and LC-IMPACT, the Global Extinction Probability is applied to combine impacts at different scales and derive CFs representing global species loss. 21 600 CFs are produced, ranging from 2.6E-19 to 1.5E-7 PDF·yr/kg (preliminary). Their application is demonstrated through LCA case studies in agriculture, textiles, and food packaging, highlighting the importance of including these impacts in LCA. Overall, the resulting CFs support environmental decision-making, are provided in different units compatible with multiple LCIA methods, and are proposed to contribute to the upcoming update of the Environmental Footprint method (EF4.0).

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5.10.P Life Cycle Impact Assessment Modelling and Application

5.10.P-Mo355 Performing life cycle impact assessment with the mid- and endpoint method ReCiPe

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While general guidelines to perform LCAs are available, specific recommendations on performing and reporting the life cycle impact assessment (LCIA) step in a standardized way are lacking. Previous LCAs have shown that this lack can lead to incomplete results, followed by misinterpretation. In the LCIA step, the magnitude and significance of the potential environmental impacts are quantified and evaluated. Here, we show how to systematically perform and report the LCIA step, to identify the most meaningful LCA results, and check their robustness. To develop the procedure, we used the widely applied LCIA methodology ReCiPe, which includes so-called characterisation factors (CFs) that express the environmental impact per unit of emission or extraction for 18 midpoint categories, such as global warming and acidification, and three endpoint categories (human health damage, ecosystem damage, and resource scarcity). The CFs are developed for three perspectives, addressing inherent value choices in the calculation models. The procedure was specifically developed to demonstrate the full use of a comprehensive multi-indicator method, making use of its full range of possibilities. We applied our method to a passenger car tire case study. Although according to the LCA guidelines, impact categories can be chosen in the goal & scope definition, we recommend to include all ReCiPe impact categories on endpoint level and all three perspectives in the initial assessment, and only decide after the contribution analyses which impact categories or areas of protection to focus on, to prevent unintended burden shifting. The most relevant impact categories could then be presented for one perspective, when all perspectives point into the same conclusion. If not, these differences should be discussed.

We argue that it is important to use the latest impact assessment methodology in LCA case studies, thus including new insights. For the future, improvements are for example expected regarding the inclusion of more toxicants, including nanoparticles and microplastics. Uncertainty in the CFs, caused by model input parameters, should also be included in a next version of the ReCiPe method, next to the scenario uncertainty embedded in the perspectives.

Being comprehensive on the LCIA results will lead to a clear, distilled message to stakeholders to decrease environmental impacts, without unintended burden shifting across the supply chain, or between different environmental impacts.

5.10.P-Mo356 How a methodology developed to identify volatile ingredients improves the reliability of impact assessment of cosmetic products in life cycle assessment

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L'Oréal has been conducting Life Cycle Assessments (LCA) to measure the environmental impacts of its cosmetic products for many years. All this knowledge has been used to create an environmental design method aiming at improving the environmental profile of products under development, while maintaining or enhancing the consumer's benefits. This method was integrated within SPOT (Sustainable Product Optimization Tool), the product evaluation tool used by product development teams to assess the environmental performance of all new Group's cosmetics. Based on a full life cycle approach, this tool is currently using the planetary boundaries concept to weight and aggregate in a single environmental footprint 14 impacts of a finished product, including formula, packaging, manufacturing and distribution to consumers.

Product portfolio analysis with SPOT showed that the formula end-of-life (i.e. after consumer use) is one of the major life cycle stages responsible for the environmental footprint of cosmetic products. However, the contribution of formula end-of life to the product footprint highly depends on the environmental compartments where formula ingredients are emitted during the product use phase. For the sake of simplification, while remaining representative of reality, most formula ingredients in SPOT are supposed to be discharged into sewers when the product is used by consumers. However, few highly volatile ingredients are released to air such as propellants and some liquid ingredients with high vapor pressure. The distinction between ingredients considered volatile and non-volatile in SPOT required the development of a specific method, as there is no harmonized and agreed-upon technical definition of a volatile chemical substance, despite the existence of several international regulations on volatile organic compounds.

The methodology developed to identify volatile ingredients for application in our product environmental design

tool SPOT will be described and its influence on the environmental footprint of ingredients, formulas and finished products will be shown through case studies. More broadly, the advantages of this methodology for the cosmetics sector and potentially other sectors wishing to improve the robustness of end-of-life assessment of chemical products in LCA will be highlighted.

5.10.P-Mo357 Evaluating the Social Impacts in the Emerging Second-Life Battery Sector: A Comprehensive Framework

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The battery sector has grown substantially over the past decade, producing an unprecedented volume of batteries to meet rising demand for sustainable energy and mobility. However, this raises concerns about battery disposal, as higher production will result in a corresponding increase in retired battery waste streams.

Second-life applications of batteries present a compelling opportunity to address this challenge. Retired batteries can be repurposed for less demanding uses like energy storage and grid balancing. This extends battery lifespan, reduces waste, decreases demand for raw materials, and promotes environmental and economic sustainability.

While the environmental and economic implications of battery reuse have been widely studied, the social dimension remains underexplored. To address this gap, this paper proposes a framework for systematically assessing the social impacts of the emerging second-life battery sector. This framework adopts a methodology aligned with the guidelines established by UNEP/SETAC and ISO 14075. The initial step involves defining the goal and scope of the assessment, with particular emphasis on establishing system boundaries and delineating which elements are included in or excluded from the analysis.

Subsequently, primary data are collected on material quantities, origins, costs, and associated processes and energy inputs to identify key social impacts. These data are complemented by databases such as PSILCA and SHDB, which allow for an evaluation of potential social impacts. Through this analysis, we can identify the main social hotspots, prioritise subcategories and indicators for further examination.

Building on the second phase of the framework, it engages directly with stakeholders through customised questionnaires and interviews with partners and sector experts. This step offers a more grounded and realistic perspective on the sector's challenges and best practices. This approach is particularly valuable for highlighting areas where early interventions can foster positive change or identifying practices that are already contributing to improved social conditions across different categories of impact.

The integrated framework contributes to a more comprehensive understanding of social sustainability within the second-life battery sector, supporting the development of strategies that enhance social value, promote responsible innovation, and strengthen overall system resilience.

Disclaimer/Disclosure:

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5.10.P-Mo358 Modelling electricity consumption for zero emissions vehicles and fleet LCAs: guidelines from the TranSensus LCA European research project

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Electricity consumption modelling is a highly debated topic in the LCA community, since the two main options that can be used: the location-based approach and the market-based approach, have both their pros and cons, and cannot be used simultaneously within the same LCA, if one wants to avoid double counting. Therefore, one of the tasks assigned to the TranSensus LCA European project, which was to reach a consensus among its consortium for a harmonised, commonly accepted and applied LCA approach for electricity consumption modelling for zero-emission vehicles and fleet, was not an easy one.

Nevertheless, due to a clear 30-month consensus building process, based on discussions, aimed at a common understanding of the location-based and market-based pros and cons, 3 consecutive voting sessions, aimed at selecting requirements and recommendations, completed by recommendations from the project Advisory Board, the following guidance was agreed upon:

- The location-based approach shall be used for the vehicles production phase. However, if desired, a 100% market-based approach can be used (in which residual mixes are used, to avoid double counting issues). Lastly, when needed, a mixed-method approach may be used. The last 2 options come with several safeguards related to the physical reality of the consumed electricity delivery.
- A “dynamic” modelling approach shall be used for the use phase, based on a reputable energy future scenario, to assess the most realistic context occurring over the full-service life of the vehicles. However, because of legal obligations, one may opt to use a more conservative “static” modelling approach, where the electricity mix at date of the vehicles production is used.

The TranSensus LCA European project lasted 30 months and included 20 beneficiaries (11 industries plus 10 academics), 24 associated partners and 4 wider consultation groups. The TranSensus guidelines, published in 2025, establish 56 methodological requirements and 81 recommendations, covering environmental and social LCAs for zero-emission vehicles, fleet and prospective scenarios analyses. It is important to note that TranSensus guidelines is a living document to be updated as necessary.

The electricity modelling guidelines may be later updated based on better electricity traceability and the availability of databases with residual mixes background processes and of new future electricity production processes (for instance from the PREMISE project).

Disclaimer/Disclosure:

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5.10.P-Mo359 Land-Use Biodiversity Characterization Factors for Wetland Agriculture in Europe **Bonivasius Pradipta Retmana, Quint van Giersbergen, Birka Wicke and Rosalie van Zelm, Radboud University, Netherlands**

Wetlands contain a large portion of the world’s soil carbon and are home to many specialized species. In Europe, however, many wetlands have been drained for agriculture, leading to high greenhouse gas emissions and biodiversity loss. Wetland agriculture, known as paludiculture has been proposed to produce biomass while maintaining ecosystem functions by keeping water tables high. However, its environmental benefits remain debated. Biodiversity in paludiculture systems is poorly documented. While biodiversity impacts due to land use are addressed in Life Cycle Assessment (LCA), paludiculture in LCA remains challenging because its species composition and land-use dynamics differ from typical agricultural systems. Popular Life Cycle Impact Assessment (LCIA) methods like ReCiPe2016 and LC-Impact, do not specifically address wetland agriculture. This research therefore aims to develop paludiculture-specific biodiversity Characterization Factors (CFs), expressing potential species loss due to paludiculture land conversion for different European countries, and compare them to CFs for drained peatland agriculture to provide impacts of paludiculture. CFs are expressed as Potentially Disappeared Fraction (PDF) of species per unit of land area. To determine CFs, species loss from land transformation and occupation were determined. Land-use areas for paludiculture, drained peatland agriculture, and natural peatlands were drawn from “Building the European Peatlands Initiative” map. Species lists and occurrence coordinates were obtained from the IUCN Red List database and the Global Biodiversity Information Facility (GBIF). All species occurrences were spatially matched to corresponding pixels in the map for each country. These inputs were used in the Species-Area Relationship (SAR) model to derive CFs for all European countries. Preliminary results for the Netherlands show that paludiculture leads to lower potential biodiversity loss compared to drained peatland used for agriculture. CFs for drained agriculture also fall within the same order of magnitude as conventional agriculture in LC-Impact, suggesting good comparability. Still, several uncertainties remain to be addressed, such as missing species in paludiculture areas due to limited spatial information and the use of a 500-year default regeneration time. As next step, we plan to expand the CF calculation to all European countries to create a spatially explicit characterization heatmap.

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5.10.P-Mo360 Modelling organic agriculture in land use impact categories: a proposal for the Environmental Footprint methods

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Currently, no consensus has emerged on methodologies to incorporate different agricultural practices (APs) into Life Cycle Assessment (LCA). This represents a major scientific challenge, whose relevance is unanimously agreed to correctly identify, assess, and thus promote, more sustainable production methods. In the context of the Environmental Footprint (EF) methods, the Joint Research Centre (JRC) has explored possible solutions to bridge this gap focusing on AP variability, in particular organic farming systems, in land use impact categories. A short-term solution has been proposed, introducing "scaling coefficients" derived from the meta-analyses available in the JRC Farming Practice Evidence Library to adjust characterisation factors (CFs), thus reflecting the effects of organic farming in the soil and biodiversity impact categories. The approach has been tested in organic datasets, showing a reduction in the impact of organic systems with limited effect at single weighted score level. The testing also unveils challenges associated to the modelling of organic farming systems, such as crop yields differences and inventory modelling, as well as co-products allocation in relation to green manure use. This proposed approach aims to enable LCA to differentiate agricultural systems while outlining future research directions. Further knowledge on the effects of organic farming on environmental indicators might enable the definition of CFs for the variety of APs, including a higher level of granularity at elementary flow level and geographical regionalisation. Better modelling of different APs might involve the revision of other impact categories, such as toxicity-related categories, or the weighting set, as well as the further development of land use impact assessment modelling to better consider local conditions (e.g. soil characteristics).

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5.10.P-Mo361 Environmental Impacts of Livestock Systems - Insights from Recent LCA Studies

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Food production, especially livestock farming, is widely recognized as a significant driver of environmental impacts worldwide. Life cycle assessment (LCA) has become a crucial approach for evaluating these systems, as it enables the quantification of environmental burdens throughout a product's entire life cycle. This work provides a systematic analysis of 54 peer-reviewed studies applying LCA to livestock production, focusing on beef, pork, chicken, lamb, and milk. The primary aim is to evaluate the consistency and comparability of methodologies across studies, as well as to identify emerging challenges and research gaps that warrant further investigation.

Results show considerable variation in reported environmental impacts, largely influenced by factors such as animal species, production practices, allocation approaches, and system boundaries. Ruminant systems, particularly beef and lamb, generally demonstrate higher global warming potential due to methane emissions and lower feed efficiency. However, these findings must be interpreted in the context of the substantial methodological differences observed among studies. Choices such as the definition of system boundaries and the selection of impact categories were found to significantly affect outcomes, limiting cross-study comparability.

Although current LCA studies provide valuable insights, they remain limited by uncertainties associated with data quality, representativeness, and the absence of standardized methods that integrate environmental, social, and economic dimensions. Future research should emphasize methodological harmonization, the development of region-specific and dynamic LCA models, and the inclusion of broader sustainability indicators. Enhancing data transparency and consistency is critical to improve the reliability and policy relevance of livestock sustainability assessments, ultimately supporting the design of more effective, context-specific mitigation strategies.

5.10.P-Mo362 Rethinking water use impact assessment: a novel multimedia fate model covering the full water balance

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Green water—soil-stored rainwater consumed by plants through evapotranspiration—accounts for the majority of agricultural water use, yet it remains largely overlooked in Life Cycle Assessment (LCA), which traditionally focuses on blue water withdrawals. This omission limits the ability of LCA to represent the full environmental implications of water use in agriculture. This study advances water-related LCA by integrating green water consumption into both life cycle inventory (LCI) and life cycle impact assessment (LCIA), while maintaining compatibility with existing blue water assessment methods. Building upon a mechanistic framework for water use impact modeling, we generate new inventory data and hydrologically consistent fate factors (FFs) that capture water stocks and flows among interconnected environmental compartments at a watershed scale.

The methodology is demonstrated for the Fluvia watershed in Catalonia, Spain, where the SWAT+ ecohydrological model was applied to simulate the spatial and temporal distribution of water resources. High-resolution outputs such as evapotranspiration, runoff, and percolation were translated into green and blue water consumption flows for major crops and agricultural practices. These data improve current LCI databases by incorporating seasonal variability, differences in irrigation strategies, and management-specific water demand. To quantify downstream impacts, we used a multimedia fate factor model representing six hydrological compartments: air, soil, vegetation, surface water, groundwater, and fossil water. Monthly transfer rate matrices (k-matrices) derived from SWAT+ outputs were inverted to compute fate factor matrices, resulting in 120 sets of FFs over a 10-year period. The inclusion of soil as an explicit compartment allows for the first time the linkage of green water consumption to its environmental consequences within LCA.

This proof-of-concept confirms the feasibility and necessity of fully accounting for water balance dynamics in LCA, enabling differentiation of water sources and their impacts at the watershed level. Future work will extend the model to broader geographic regions, allowing the identification of agricultural practices that reduce water consumption and associated impacts.

5.10.P-Mo363 Towards an Impact Assessment of Land Use on Soil Biodiversity

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Life cycle impact assessment (LCIA) indicators assessing land-use impacts on community composition are currently limited to a few well-studied groups, overlooking soil organisms despite their major contribution to global biodiversity and ecosystem functioning. Advances in environmental DNA (eDNA) metabarcoding and large-scale monitoring now provide the data needed to incorporate soil organisms into these models and improve the taxonomic coverage of biodiversity impact assessment. Relative change of species richness (PDF) is commonly used in LCA to estimate damage to biodiversity. For microbes, richness can increase with land use intensity, leading to counterintuitive results in LCA, such as a beneficial effect of land conversion for local soil biodiversity. A more detailed analysis of lower taxa affinities (phyla, class) with land use is then required to transcribe insights from soil ecology into LCA. Methodological adaptations are also required to derive community composition indicators from environmental DNA data rather than species inventories. Two major eDNA data collection programs are now available to test our methodology: RMQS, in France, focusing on bacteria, and LUCAS Soil, across Europe, focusing on bacteria, fungi, protists and eukaryotes. Processing robust soil biodiversity characterization factors requires addressing key methodological consideration. Taxonomic richness for microorganisms is estimated via OTU richness, with definitions of OTUs (e.g., 95% similarity, zero-radius) influencing richness estimates. The choice of the taxonomic rank affects land-use affinities, as bacterial and fungal richness patterns vary across phyla and classes, making averaged subtaxa characterization factors different from kingdom level ones. Spatial aggregation shows that ecoregions, commonly used for plants and vertebrates, may mask fine-scale variation in bacterial richness, which is strongly influenced by soil properties such as acidity, texture, and C:N ratio. Finally, reference situations need to be defined to compute PDF, but samples from natural sites are scarce, especially considering the needed representativeness for all pedoclimatic regions. Reclassification of sites under human land use could be performed following conventional approaches. Used on large scale datasets (RMQS, LUCAS), this methodology will be used as a LCIA model to assess the impacts of land use on below-ground biodiversity, complimentary to models for above-ground biodiversity.

5.10.P-Mo364 Framework development for characterization factors of marine biodiversity assessment under future climate change.

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The ocean covers more than 70% of the Earth's surface and supports a very large amount of biodiversity, estimated to be over 80% of all global species. Recent studies have shown that climate change is creating serious challenges not only for life on land but also for marine ecosystems. Changes such as increasing sea temperature, ocean acidification, and deoxygenation are disrupting the stability of marine environments and may lead to strong reductions in fisheries and habitat quality. However, in the LCIA field, research on marine biodiversity is still very limited, especially regarding the development of characterization factors (CFs) for marine species.

In this study, we propose a practical framework to develop spatial characterization factors for marine biodiversity under future climate change, mainly focusing on sea temperature rise. We follow the LIME3 approach and combine it with species distribution models (SDMs) to estimate changes in species habitat suitability under future scenarios. Environmental data for both current conditions and the 2100s (SSP1–RCP2.6) were taken from Bio-ORACLE, including variables such as ocean temperature, salinity, and nutrients. Occurrence records of marine species were collected from GBIF and OBIS.

The resulting CFs are shown on maps with a 0.05° resolution and expressed as the marginal effect of greenhouse gas emissions on species extinction rate, following the indicator recommended in the Planetary Boundaries framework. These CFs help identify which species are more vulnerable to future climate change. They also provide a way to check whether different climate mitigation policies or emission reduction scenarios are strong enough to protect marine ecosystems. For LCA users, this framework makes it possible to quantify life-cycle impacts on marine species and also compare these results with existing terrestrial assessments. Overall, this research helps connect ecological information with LCIA and contributes to filling the current gap in marine biodiversity characterization factors.

5.10.P-Mo365 Integrating Soil Biodiversity Impacts of Open Trenching into Life Cycle Impact Assessment

Monu George Varghese, INaB, RWTH Aachen, Germany

Soil biodiversity plays a central role in maintaining ecosystem functions, yet disturbance from open trenching remains poorly represented in Life Cycle Impact Assessment (LCIA). Existing practice relies primarily on the Potentially Disappeared Fraction (PDF) of species as an indicator of biodiversity loss, which captures direct habitat disruption but does not reflect fragmentation effects or post-disturbance recovery. This limits the ability of LCIA to characterize the full ecological consequences of excavation activities.

This work addresses the challenge by developing a demonstrative framework that retains PDF as the main indicator while testing how fragmentation metrics and recovery dynamics can be integrated to improve the assessment of soil biodiversity impacts from open trenching. The purpose is to explore methodological options, evaluate their feasibility, and identify where existing LCIA models can be strengthened.

The approach uses literature-derived characterization factors, soil and habitat data, and spatial fragmentation indicators such as edge density and connectivity. Biodiversity loss is quantified using standard PDF calculations and then adjusted through fragmentation effects and recovery scenarios based on soil return after excavation.

This allows the comparison of baseline PDF-only assessments with extended versions that incorporate ecological structure and temporal recovery. Intermediate results focus on the extent to which fragmentation amplifies impacts and how soil restoration influences recovery potential.

Preliminary findings suggest that including fragmentation and recovery dynamics alters both the magnitude and distribution of biodiversity impacts, revealing where recovery pathways can mitigate disturbance and where fragmentation leads to persistent ecological degradation. These outcomes highlight methodological opportunities for improving the representation of soil biodiversity in LCIA and offer a basis for future development of more ecologically realistic indicators.

The study contributes to ongoing efforts to strengthen biodiversity modelling within LCIA and supports more informed decision-making in infrastructure and environmental planning.

5.10.P-Mo366 Assessing Biodiversity with LCA: How far can we go?

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Biodiversity loss is one of today's major environmental challenges. In this context, Life Cycle Assessment (LCA) has long sought to quantify impacts on biodiversity through the "ecosystem quality" area of protection. To help industrial stakeholders better understand the capabilities and limitations of these approaches, SCORE LCA has launched a study on the relationship between LCA and biodiversity, aiming to provide both state-of-the-art and practical recommendations.

Two case studies serve as illustrative threads: electricity generation from photovoltaic systems in France, and the ecological restoration of an industrial site after solar farm decommissioning. These examples help examine how existing LCA impact assessment methods cover biodiversity pathways, how their metrics should be interpreted, and where their blind spots lie. The study compares major LCIA methods by analyzing their coverage of biodiversity loss drivers, the meaning and relevance of their metrics, their geographical resolution, and their alignment with global biodiversity monitoring concepts and frameworks.

Current LCIA approaches only partially capture the five key biodiversity loss drivers. They also rely on metrics with differing meanings, making interpretation challenging. Moreover, they struggle to reflect local ecological conditions, land-use practices, or pressures such as invasive species. The case studies aim to clarify how these issues affect real-world assessments, including the difficulty of addressing restoration and compensation through LCA, and the limited integration of biodiversity variables.

Beyond reviewing methods, the study will deliver practical recommendations. It will help practitioners understand when LCIA is appropriate for biodiversity evaluation, when complementary tools are necessary, and how LCA fits within emerging corporate sustainability frameworks. Completion is expected in March 2026 and will provide guidance on combining LCA with other biodiversity indicators to support robust decision-making.

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5.10.P-Mo367 Assessment of the impact of nitrogen deposition on terrestrial ecosystems

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Global biodiversity is declining at an unprecedented rate. According to IPBES, at least 680 vertebrate species have gone extinct since 1500, and the current extinction rate is tens to hundreds of times higher than the long-term average over the past 10 million years. Approximately 25% of assessed taxa, including plants, are listed as threatened on the International Union for Conservation of Nature and Natural Resources (IUCN) Red List. The planetary boundaries framework further indicates that biogeochemical flows have substantially transgressed safe limits, with nitrogen identified as a major contributor. Although nitrogen is an essential nutrient for plants, increased anthropogenic nitrogen emissions enhance nitrogen deposition, altering plant nitrogen use, productivity, and community composition. However, the quantitative relationship between nitrogen deposition and plant biodiversity loss remains insufficiently resolved.

The objective of this study is to quantitatively evaluate the effects of nitrogen deposition on terrestrial plant biodiversity. We estimated the Mean Species Abundance (MSA), a biodiversity indicator representing species richness and relative abundance compared to undisturbed natural conditions, using global nitrogen deposition data for 2010 at a spatial resolution of $1.125^\circ \times 1.125^\circ$. The deposition dataset includes dry and wet deposition of both ammonium and nitrate ions. MSA values were calculated following the methodology of Hans van Grinsven et al., focusing on vascular (higher) plants at the global scale.

Visualization and country-level comparison of nitrogen deposition using QGIS reveal that China, the United States, India, and Russia exhibit the highest nitrogen deposition impacts. This pattern is reflected in the MSA estimates, suggesting substantial risks to plant biodiversity in these countries. By partitioning the globe into 32 source regions and using scenario data for additional NH_3 and NO_x emissions from each region, it is possible to identify which emitting regions contribute to deposition in which receptor regions.

This study provides a quantitative basis for relating nitrogen deposition to plant biodiversity decline and for comparing impacts among countries and regions. In future work, we will extend the approach to assess how changes in plant biodiversity propagate to broader biodiversity and ecosystem service impacts.

5.10.P-Mo368 How to Integrate Marine Environmental Impacts of Marine Technologies into Life Cycle Assessment

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Human activities are increasingly expanding into marine environments. While Life Cycle Assessment (LCA) is used to evaluate marine technologies environmentally, it faces challenges in accounting for marine-specific impacts. Advancing LCA for marine technologies requires a clear understanding of their interaction with the marine environment, which is essential for developing (sub-compartmentalised and regionalised) characterisation factors (CFs). In our study, we first present a methodological approach for identifying pathways for marine environmental impacts, which includes qualitatively determining the marine environmental flows and the fate, exposure, and effect processes of the marine technology, as an initial step toward CF development. We then demonstrate its application using ocean alkalinity enhancement (OAE) as a case. OAE is a marine carbon dioxide removal technology that is attracting growing interest due to its potential contribution to climate mitigation targets. Our approach draws on oceanography and geochemistry literature relevant to the technology, and is complemented by expert input from the fields of life cycle impact assessment (LCIA), oceanography, and OAE. We analyse which components of the marine environmental impact pathways are currently modelled in LCIA and identify missing elements, key knowledge gaps, and research needs to improve OAE impact assessment within LCA. From our analysis, three main impact categories emerge for OAE marine environmental impacts: marine ecotoxicity, marine eutrophication, and ocean acidification. Current LCIA models do not capture these pathways entirely and necessitate significant adaptation for OAE evaluation. Present models lack relevant environmental flows, represent the ocean in a simplistic manner that neglects its spatial variability, and often assume emissions entering the marine environment via freshwater or atmospheric systems rather than direct ocean release. Future research must develop OAE-specific fate models, expand experimental data on ecotoxicological and eutrophic responses to OAE deployment, and assess the effect of enhanced alkalinity on a wider range of calcifying species. Models incorporating marine sub-compartments must also be developed for spatially accurate impact assessment. Overall, our methodology provides a structured approach to identifying marine environmental impact pathways of marine technologies and can also be more broadly applied to other emerging technologies.

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5.10.P-Mo369 Life Cycle Impact Assessment of dynamic fisheries: Characterization factor temporal series for Peruvian stocks

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Recently developed Life Cycle Impact Assessment (LCIA) models offer a promising framework for quantifying the environmental footprint related to the exploitation of biotic resources. The Fisheries Impact Pathway proves to be a reliable LCIA model for quantifying these impacts; however, we have identified certain methodological aspects relevant to impact assessment in dynamic fisheries, particularly temporality, that require enhancement to better capture biodiversity impacts. This study aims to develop a characterization factor (CF) temporal series for 10 representative Peruvian fishing stocks. The methodology framework comprises 1) improving model performance using extensive statistical criteria to obtain reliable estimates for CF calculation; 2) implementing an exclusive economic zone (EEZ) approach to address the efficiency of national policy interventions; and, 3) integrating time variability in stock abundance, which is relevant for stocks affected by El Niño Southern Oscillation (ENSO).

According to our statistical analysis, improved model estimates result when abundance indices are included within a narrower modelling timeframe. The refined CFs calculated using these estimates deviate up to 4 orders

of magnitude from previously reported values, usually showing lower values, reduced uncertainty and pronounced sensitivity to annual biomass fluctuations. In addition, weak to moderate statistical correlations were observed between the CFs and typical ENSO indices, suggesting sensitivity to climatic events driving stock landings and biomass variability. Furthermore, the case studies revealed differences in biodiversity impacts across stocks destined for direct human consumption, as well as additional impacts, up to 61% in fishmeal and fish oil production among plants operated by a major local producer. These findings indicate that the temporal dimension of fishery-related CFs is critical, especially when fluctuating parameters are intrinsic to the modeled system. We recommend applying these dynamic CFs rather than the static CFs to quantify marine biotic resource depletion impacts for Peruvian seafood and aquaculture.

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5.10.P-Mo370 Which Area of Protection for Fish?

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Fishery impacts remain insufficiently covered in current LCA studies. To address this gap, the European Commission tasked experts with integrating fisheries into the Environmental Footprint method. Comparing the available approaches helps clarify the distinction between impact and characterisation factor (CF), and between protecting ecosystems (Ecosystem Quality area of protection (AoP)) and preserving ecosystem services (Socio-economic assets). Two main approaches were examined. The fisheries science approach developed by the Scientific, Technical and Economic Committee (STECF) evaluates stock status using two indicators: abundance (current biomass relative to a reference) and fishing pressure (mortality relative to a reference). These feed a decision tree, with impacts increasing as pressure rises and abundance decreases. The LCA approach derives CFs from a dynamic population model. It integrates abundance, pressure, resilience (replenishment rate) and stock size. High pressure increases impacts, while low abundance, resilience or stock size decreases them. Missing values can be replaced by modelled ones. Both approaches depend on abundance and pressure, but differ in important ways. STECF assumes no impact before optimum exploitation is exceeded, whereas LCA considers impacts from the start of fishing. LCA also adds a temporal dimension through resilience. Most importantly, STECF describes the state of the stock, while LCA quantifies the responsibility of a specific fishing activity for that state, which explains the inclusion of stock size in LCA. As a result, the two frameworks may diverge significantly. These differences raise a central question: should assessments focus on the intrinsic ecological value of fish populations or on their instrumental value as resources? For the Ecosystem Quality AoP, LCA is more suitable because it treats all species as equally important and evaluates impacts proportionally. For the Socio-economic assets AoP, the STECF approach is more aligned, since larger stocks provide greater resource value. Both approaches can also be adapted to either perspective simply by adding or removing stock size in CF calculations. The author concludes that the LCA approach offers stronger methodological consistency, while STECF benefits from acceptance within fisheries science. Ultimately, both perspectives are needed for integrating fisheries into the Environmental Footprint method.

5.10.P-Mo371 Incorporating Black Soldier Fly oil in Aquafeeds: Environmental Implications from a Life Cycle Perspective

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Fishmeal (FM) and fish oil (FO) are the primary protein and lipid sources in aquafeeds, widely used for their high nutritional quality and essential nutrients for optimal fish growth. However, their production is linked to the overexploitation of marine resources and related environmental impacts. Alternative ingredients, such as those derived from black soldier fly (BSF), offer a sustainability opportunity by converting agri-food by-products into BSF protein meal and oil. BSF oil, due to its rich nutrient profile (particularly fatty acids), can support fish health and performance.

Using life cycle assessment (LCA) methodology, this study assesses and compares, from a cradle-to-gate perspective, the environmental performance of a conventional aquafeed and an innovative one that partially

replaces FO, both used for seabass production. The functional unit is 1 tonne of aquafeed. Primary data was collected from a representative Portuguese aquafeed producer, and the impact assessment was conducted using the Product Environmental Footprint.

Preliminary results reveal that aquafeed containing BSF oil performed worse in climate change (CC) and freshwater eutrophication (FE) but delivered improvements in marine eutrophication (ME) due to reduced sunflower meal impacts, with minor gains observed for acidification (AC) and fossil resource use (FRU). The farming and diet of BSF larvae influenced CC and FE outcomes, highlighting the need for production optimisation. Feed ingredient production was the hotspot, accounting for over 90% of CC, FRU, and FE impacts in innovative aquafeed, compared to 84% of CC and 96% of ME in conventional aquafeed. Cereals and by-products were the main contributors, with wheat, maize, and pea production driving AC and EU. In contrast, energy use and fuel (mainly steam from natural gas and diesel for agricultural machinery) dominated CC and FRU impacts. Transportation of feed materials also contributed substantially, higher than 10% to the impact for CC, AC, and FRU.

Overall, partially replacing FO with BSF oil does not appear to yield significant improvements in aquafeed sustainability, underscoring the importance of locally sourced feed ingredients. Further research, by expanding system boundaries to include farm-gate performance and economic and social dimensions, will support a more holistic sustainability assessment and evidence-based decision-making for sustainable aquaculture.

5.10.P-Mo372 An updated characterization model for freshwater consumption impacts on riverine fish species in LCA

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Consuming freshwater beyond the regional carrying capacity—the maximum volume of water that can be sustainably used by human activities—poses a critical threat to aquatic biodiversity and ecosystem integrity. Therefore, quantifying the potential impacts of such overconsumption is essential for guiding responsible and sustainable water use and for assessing the environmental impacts of global value chains. In Life Cycle Assessment (LCA), these impacts have typically been assessed through Life Cycle Impact Assessment (LCIA) frameworks, which develop spatially explicit characterization factors to quantify the potential damage to freshwater ecosystems caused by human water use. However, existing approaches generally treat water use as an undifferentiated pressure, without distinguishing between sustainable consumption and overconsumption beyond regional carrying capacity. In this study, we quantified the regional carrying capacity of freshwater consumption as the difference between available freshwater resources and the environmental water requirements needed to sustain aquatic ecosystems across approximately 11,000 watersheds worldwide, based on WaterGAP model. We then identified freshwater overconsumption by human activities and applied a global model relating freshwater fish species richness to river discharge and other covariates (elevation, basin area, and climate zone) to derive two species–discharge relationships (SDRs), distinguishing watersheds experiencing overconsumption from others. These SDRs were subsequently used to develop characterization factors measuring the effects of human freshwater consumption on freshwater fish biodiversity. Our analysis revealed higher characterization factors in watersheds where human water consumption exceeded regional carrying capacity, indicating stronger biodiversity impacts under overconsumption conditions. These findings highlight the importance of explicitly accounting for overconsumption in LCIA water-impact assessment models. Incorporating this distinction can improve the accuracy of global characterization factors and support more responsible and targeted freshwater management strategies aligned with biodiversity conservation goals. The model integrates LCIA consensus methods from the UNEP Life Cycle Initiative to enhance the assessment and make the biodiversity impacts more consistent with the midpoint impact assessment method “AWARE”.

5.10.P-Mo373 Do antibiotics have a major impact on aquatic wildlife? A freshwater ecotoxicity impact assessment of antibiotic mixtures including binary interactions

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Every year, significant amounts of antibiotics enter the environment through human consumption in households and hospitals or use in the veterinary sector. These substances cannot be fully removed via wastewater treatment. Consequently, antibiotics end up in surface waters where they can affect freshwater ecosystems. The European Commission recently launched a revised version of the Urban Wastewater Treatment Directive to reduce environmental damage from pharmaceutical emissions. A good understanding of the freshwater

ecotoxicity impact caused by these chemical mixtures is needed to encourage the protection of aquatic wildlife.

Life cycle assessment (LCA) places ecotoxicity in a broader perspective by looking at the environmental impact over the entire life cycle. Traditionally, the USEtox model is utilized within LCA to calculate the ecotoxicity impact of chemical substances. To quantify the impact of a chemical mixture, the impact of all individual substances is simply summed up. However, interactions between substances in the mixture are not taken into account. In the TransPharm project, a novel methodology was developed to determine the ecotoxicity impact of chemical mixtures on freshwater ecosystems.

In the new methodology, concentration addition was applied at species level to create a model that is consistent with the original concentration addition concept derived from environmental risk assessment. Next, binary interactions are taken into account using the cumulative risk assessment framework from the Environmental Protection Agency in the United States. This new method was demonstrated based on a Swiss hospital that discharges antibiotics.

The results from this assessment show that the new model mostly leads to a lower freshwater ecotoxicity impact of an antibiotic mixture compared to the traditional model. For example, the ecotoxicity impact of a norfloxacin-erythromycin mixture is overestimated by 23% when using the traditional model. By including binary interactions, the ecotoxicity impact slightly increases (4% for norfloxacin-erythromycin mixture) or decreases (4% for norfloxacin-ciprofloxacin mixture) depending on the considered mixture. A sensitivity analysis illustrates that the impact on freshwater ecosystems is highly dependent on the included interaction parameters. This impact can vary by up to 400%. Nevertheless, this work concludes that including interactions may unnecessarily increase the complexity of these calculations.

Disclaimer/Disclosure:

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5.10.P-Mo374 New and robust freshwater ecotoxicity characterization factors for pharmaceuticals toward a more complete end-of-life evaluation

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Pharmaceuticals are biologically active and diverse compounds that are not fully removed in wastewater treatment and can reach surface waters. Their specific mode of action may affect non-target species, yet assessing their environmental relevance remains challenging due to limited ecotoxicity data.

Life cycle assessment (LCA) quantifies the environmental impacts of products and services. In the Life Cycle Impact Assessment (LCIA), the magnitude 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 impact categories. The USEtox model, the consensus LCIA model, contains 3104 substances, but only 67 are pharmaceuticals. Thus, many pharmaceuticals are treated as having no ecotoxicity impact in LCIA calculations, leading to underestimation in LCIA results. This study aimed to increase the number of pharmaceuticals included in ecotoxicity assessment by deriving CFs for freshwater ecotoxicity. We derived CFs for 292 pharmaceuticals, 21 of which were compared with the existing CFs in USEtox. For 60 pharmaceuticals, we compared EFs derived from EC10 values extrapolated from acute EC50s versus chronic NOECs. A case study was conducted to demonstrate the applicability of the new CFs in LCA. Input data for fate and exposure factors were compiled from multiple databases. Effect factors (EFs) were derived from chronic EC10s collected from different databases for at least three trophic levels using HC20 as a benchmark. The new CFs were applied in a case study quantifying freshwater ecotoxicity from pharmaceuticals discharged with treated municipal wastewater. UBA emission data were combined with both new and existing USEtox CFs to calculate impact scores.

The largest CFs were obtained for pharmaceuticals with a highly specific mode of action, such as ethinyl estradiol. For such pharmaceuticals, EFs based on chronic EC10s extrapolated from chronic NOECs can be several orders of magnitude higher than those based on chronic EC10s extrapolated from acute EC50s, showing that acute-to-chronic extrapolation factors for crustaceans and fish may underestimate chronic effects. In the case study, impact scores were about seven times higher with the new CFs than with USEtox CFs. By

substantially expanding ecotoxicity coverage, this study enables a more complete evaluation of pharmaceutical emissions in healthcare LCA studies.

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5.10.P-Mo375 Integrating Ecotoxicity Testing and Life Cycle Assessment (LCA) for Holistic Evaluation of Advanced Wastewater Treatment

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A comprehensive evaluation of wastewater treatment processes requires analytical frameworks capable of capturing both the ecotoxicological effect of treated effluent and the system-wide environmental footprint of the treatment systems. Ecotoxicological assays, with untreated and treated effluents, provide critical insights by measuring biological responses to complex mixtures, revealing additive, synergistic, and antagonistic effects. On the other hand, Life Cycle Assessment (LCA) offers a structured approach to quantify environmental impacts across all operational stages, including emissions to air, water, and soil. Integrating these tools can provide a more holistic approach to assessing the environmental impacts of advanced wastewater treatment technologies designed to remove contaminants of emerging concern, for which chemical analyses alone may be insufficient.

This review critically examines methodological pathways for combining standardized ecotoxicity testing with LCA to characterize the environmental impacts of conventional and advanced wastewater treatment processes. Case studies involving activated sludge, moving bed bioreactors (MBBRs), ozonation, and granular activated carbon treatment processes are included to illustrate how the integration of ecotoxicological assays with LCA can provide a comprehensive evaluation of the benefits of advanced wastewater treatment processes. The use of a multi-species test battery to assess toxicity and provide insights into the potential risks of transformation products formed during treatment is also explored. These practical examples also highlight persistent challenges, including variable sensitivity among test organisms, limited data for chronic and sublethal effects, and a lack of holistic evaluations across diverse micropollutants.

In conclusion, this integrated approach strengthens the scientific basis for evaluating and optimizing advanced wastewater treatment technologies and enhances the capacity to support more environmentally protective decision-making.

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5.10.P-Mo376 Exploring Ecotoxicity Impacts of Plastic Additives in Fishing Gear and Implications for LCIA

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Plastic waste in marine environments is a growing concern, with approximately 20% of ocean plastic pollution originating from the fishing and aquaculture sectors. This research addresses a critical gap in life cycle impact assessments (LCIA) by investigating the ecotoxicity of plastic additives found in lost fishing gear, which are often overlooked despite their potential harmful effects on marine ecosystems. The purpose of this study is to elucidate the ecological impacts of these additives and to improve existing LCIA methodologies.

To tackle this challenge, we conducted a comprehensive assessment using the Norwegian fishing sector as a case study. We adopted the ISO 14040:2006 standard and employed the USEtox method to systematically evaluate the impacts of various fishing gear types, including trawl nets, gillnets, autoline, demersal seine nets, and traps and pots. Our methodology involved calculating the emissions of plastic additives into marine environments based on their concentrations and the loss rates associated with gear components. We developed

two scenarios: a baseline scenario including only confirmed gear components, and a secondary scenario that included potential undocumented additives, leveraging Monte Carlo simulations to account for uncertainties.

Preliminary results indicate that incorporating additional components substantially increases ecotoxicity impacts across all gear types. Specifically, traps and pots exhibited the highest baseline impacts, primarily due to their low capture rates. Sensitivity analyses highlighted the critical role of additive leakage and bioavailability in determining overall environmental impacts.

Our findings underscore the urgent need for more comprehensive data on plastic additives in life cycle inventories, which currently capture less than 10% of relevant information. By advancing the understanding of the ecotoxicological effects of plastic fishing gear and enhancing LCIA methodologies, this study highlights the pressing need for better regulatory frameworks and mitigation strategies within the fishing industry, ultimately promoting sustainable practices and reducing marine pollution.

5.10.P-Mo377 Integrating The Cocktail Effect In Life Cycle Assessment

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Life Cycle Assessment (LCA) currently evaluates toxicity impacts using characterization factors that assume additive effects between substances. This simplification overlooks potential interactions within chemical mixtures, creating uncertainty about whether impacts are under- or overestimated. Recent advances in mixture risk assessment underline the need to integrate combined effects into environmental models. In this study, we test the LCA additivity assumption and develop a proof of concept that explicitly incorporates chemical mixture (“cocktail”) effects into effect-factor calculations.

We investigated contaminants frequently detected in French urban wastewater, including pesticides, metals, pharmaceuticals, and other compounds using *Gammarus* spp. as a model organism. Each substance was tested individually across a concentration gradient reflecting environmental levels up to 50× their maximum effluent concentration. Binary and multi-substance mixtures were then assessed at different concentration combinations. Monosubstance data were used to generate Independent Action (IA) and Concentration Addition (CA) predictions for mixture toxicity.

Observed mixture effects were compared to IA expectations to determine whether responses were additive, synergistic, or antagonistic. For mixtures showing deviations, alternative modelling strategies were applied. We then fitted the mixture toxicity data using a Generalized Additive Model (GAM), incorporating nonlinear dose-response functions and an interaction term capturing the nature of mixture effects. From the GAM-derived response surfaces, we extracted mixture EC10 values of one substance at fixed proportions of the other and compared them to monosubstance EC10 values to compute an allocation factor (α). Assuming similar patterns across species, we extrapolated these results to ecosystem-level sensitivity and recalculated hazardous concentrations (HC20) for mixtures. This allowed us to define a cocktail effect factor that adjusts the LCA Effect Factor, producing more realistic thresholds that reflect exposure to multiple co-occurring contaminants. This work introduces a new modelling framework integrating mixture effects into LCA toxicity characterisation. The approach will be applied to case studies of water bodies impacted by urban and industrial effluents and may support improved prioritisation of substances for wastewater treatment based on both concentrations and mixture toxicity potential.

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5.10.P-Mo378 The Updated and Consolidated ProScale-E - Ecotoxicity Potential Scoring Method for Chemical Footprint, LCA and SSbD assessment

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This abstract presents the updated ProScale-E version (2.0). The ProScale-E method has now been revised in order to align the ProScale and ECETOC Task Force approaches. Novel results from the update are shown in this presentation. The ambition for the current ProScale-E model was to a) obtain a model aligned with REACH,

straightforward to use, easy to understand, require little to moderate substance data and still provide meaningful results for substances classified according to ecotoxicity (hazard classes in the H400 family) thereby accounting for the severity of impacts, b) accounting for potency of substances, and c) facilitating inclusion of fate and degradation of substances.

A detailed analysis was carried out of the criteria for hazard classification of ecotoxicity.

The consolidated scoring system for eco-toxicity hazards based on a grouping of hazard statements from CLP is aligned with REACH classification. Assigning significance to the M-factor is a key aspect of ProScale-E, as the M-factor is based on EC50 values, and therefore introduces the potency of a substance into the scoring.

Substances having more than one H4XX-phrase (e.g. classified as both acute and chronic) will be assigned to the highest ProScale-E hazard class among the ones relevant to the classification for that substance. ProScale-E accounts for degradation in three levels, each separated by a factor of 10: 1) readily degradable, 2) rapidly (but not readily) degradable/inherently degradable, 3) not degradable/persistent/very persistent.

Comparing ProScale-E 2.0 with its predecessor, it is evident that the update is relevant, due to the span of ratios (Table 1) for even this small sample of substances. As an example, Bisphenol-A (CAS: 1980-05-07) comes out with the same characterization factor (CF) for ProScale-E 1.0 and 2.0, whereas TDI (CAS: 584-84-9) has a factor 1000 lower CF in version 2.0 compared to version 1.0.

ProScale-E appears to be an easy-to-use approach for screening level ranking/prioritization of ecotoxicity impact potentials. Development of characterization factors and validation is in progress, as is application in actual assessments.

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5.10.P-Mo379 Local-Scale Toxicity Impacts in USEtox: Assessing Variability Across Selected Emission Scenarios

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Life Cycle Impact Assessment (LCIA) models often rely on site-generic approaches and average assumptions when quantifying characterization factors (CFs), reflecting the holistic scope of LCA and limited availability of spatially resolved inventory data. Numerous studies have explored ways to incorporate region-specific conditions to enhance model accuracy and reduce CF uncertainty. For human toxicity and freshwater ecotoxicity, the UNEP/SETAC reference model USEtox already enables partial spatialization through sub-continental archetypes and an urban air compartment for densely populated areas. More recently, the inclusion of a nested, multi-compartment local scale in the USEtox model was proposed, quantifying the potential over- or underestimation of CFs when emissions occur locally rather than at continental scale. In our study, we apply the USEtox model with the proposed local-scale extension to investigate the variability of human toxicity and freshwater ecotoxicity CFs for a broad range of site-specific urban emission scenarios. We model emissions of multiple toxic chemicals across 10 European urbanized areas with different meteorological and exposure conditions. Temperature, wind speed, rainfall rate, and air mixing height were sourced from Copernicus ERA5 reanalysis dataset, while exposure conditions reflect partially urbanized environments including freshwater, natural soil, and agricultural soil compartments. The resulting CFs are used to (i) evaluate and discuss the modifications introduced to incorporate the local scale in LCIA toxicity and ecotoxicity characterization for conditions where local-scale emission data are available, (ii) quantify the influence of local meteorological and exposure parameters on CF variability, and (iii) compare local-scale CF variability with that derived for different sub-continental regions. For both human toxicity and freshwater ecotoxicity, CF variability for emissions to local air, freshwater, and soil remains within one order of magnitude. The largest variability occurs for emissions to natural and agricultural soils, particularly for substances such as benzene and toluene, for which the highest local CFs are associated with scenarios characterized by relatively high annual rainfall. Overall, these findings support a more accurate representation of impacts of toxic chemical emissions in sub-regional contexts, while balancing the search for spatial resolution with data availability and practical application.

5.10.P-Mo380 Early-Stage Life Cycle Assessment to Support the Ecodesign of Laboratory-Scale Poly(hydroxyalkanoate) Production Strategies

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Fossil-based plastic pollution and the growing burden of food waste management highlight the need for more sustainable material systems. Among emerging solutions, bioplastics such as poly(hydroxyalkanoate) (PHA) offer potential advantages, but their environmental performance depends strongly on feedstock origin and bioprocess configurations.

The BioLaMer project, funded by the European Innovation Council, investigates a proof-of-principle fly larvae biorefinery in which black soldier fly (BSF) larvae feed on food waste, from which lipid-rich fractions are recovered and used for PHA. The challenge addressed in this study is to evaluate whether early-stage Life Cycle Assessment (LCA) can support ecodesign by distinguishing environmental trade-offs between alternative laboratory-scale strategies.

To address this question, PHAs were produced by cultivation of the bacterium *Cupriavidus necator* on BSF larvae lipid-rich fraction as the sole carbon source. A gate-to-gate LCA is being performed to compare two cultivation scenarios in 3-L bioreactors operated for 36 h or 60 h. The system included inoculum preparation, microbial growth under varying nitrogen and lipid concentrations, PHA accumulation, and finally enzymatic extraction of the biopolymer. Foreground data were obtained from laboratory experiments, while background data were sourced from Sphera and Ecoinvent databases. Environmental impacts will be assessed using the Environmental Footprint 3.1 method.

With the experimental inventory now complete, LCA modelling is underway. The assessment will therefore test how early-stage LCA can identify meaningful environmental hotspots and operational trade-offs at a stage where process modifications remain feasible and cost-effective.

The findings of this study, integrating LCA into low-TRL (Technology Readiness Level) bioprocess development, aim to inform the ecodesign of PHA production pathways within BioLaMer project and to demonstrate whether early environmental assessment can guide more sustainable decision-making before scale-up.

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5.11 Modelling of Environmental Footprints of Critical And/Or Strategic Raw Materials

5.11.T-01 The Critical Raw Materials act: exploring criteria to prioritise critical and strategic raw materials

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Several industries in the European Union (EU) heavily rely on critical raw materials (CRMs), being indispensable inputs for a wide set of technologies and applications. However, the EU's demand for CRMs is mostly met by imports, exposing the EU's strategic dependencies to potential geopolitical shifts and crises. The Joint Research Centre (JRC) is supporting the implementation of Article 31 of the EU Critical Raw Materials Act (CRMA) by performing an analysis to assess the necessity and proportionality for introducing an Environmental Footprint Obligation (EFO) of CRMs. To assess which CRMs should be prioritised for EFOs, the present study focused on the analysis of the type and scale of environmental impacts of CRM consumption in the EU27 for 2022. Numerical findings associated with the life cycle environmental impacts of the CRMs (calculated for 16 impact categories of the EU Product Environmental Footprint) were combined with data on consumed masses (both domestically produced and imported) leveraging Material System Analysis studies. Stakeholders and experts were actively involved during the whole development of the project and contributed by providing key data. The results of this study underlined the key role of aluminium, copper and manganese as

dominant drivers of environmental impacts across various impact categories. Overall, aluminium occupied the first position out of the SRMs analysed in 13 scrutinised impact categories. Aluminium and copper, together with manganese, occupied the three top positions in the rankings in more than 66% of the impact categories tested. On top of the magnitude of the environmental footprints of the CRMs and SRMs, the exploration of additional criteria (such as co-product dependency, criticality and strategic relevance) showed that a holistic approach to prioritising raw materials is advisable. For instance, Platinum Group Metals (PGMs) exhibit a higher economic importance than aluminium, despite having lower total environmental impacts. Even if the analysis of the environmental footprint associated with each SRM represents an important means to assess the achievement of CRMA Article 31 objectives, the findings related to the prioritisation criteria analysis suggest that the identification of a ranking of SRMs should not only examine closely their environmental impacts, but should also include other relevant criteria that may coincide with, or differ from, the assessment of environmental sustainability impacts.

5.11.T-02 Prospective Life Cycle Assessment to Support Decarbonization of Critical Raw Material Supply Chains

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The mining and metals sector faces the dual challenge of scaling up production to meet growing demand while simultaneously reducing greenhouse gas (GHG) emissions and other environmental impacts. Critical raw materials (CRMs) underpinning clean energy technologies are under increasing scrutiny to avoid unintended burden shifting toward resource extraction and refining. Life cycle assessment (LCA) has been widely applied to assess CRM supply chains, yet its potential to guide decarbonization strategies remains underexplored. Prospective LCA extends the capabilities of LCA by integrating future scenarios to anticipate environmental implications of technological innovations, policy developments, and market shifts. Here, we showcase scenario analysis and prospective LCA to inform decarbonization strategies for CRM supply chains through two complementary approaches: i) evaluating the technical mitigation potential and trade-offs of decarbonization options and ii) assessing future scenarios aligned with climate policies and industry roadmaps. For the first approach, we focused on four CRMs used in lithium-ion batteries: lithium hydroxide, cobalt sulfate, nickel sulfate, and graphite. An attributional LCA identified GHG hotspots, based on which we quantified the mitigation potential of a scenario involving renewable electricity and electrified heat for on-site operations, energy efficiency, renewable electricity and electrified heat in the chemical industry, and renewable electricity elsewhere in the global economy. Combined, these options could reduce GHG intensity by 53–86%, leaving a mitigation gap of 14–47% even under such optimistic scenario. The second approach accounts for plausible future developments in technology and policy, providing a more plausible perspective on the achievable mitigation potential. Here, we focused on 24 operating and announced projects producing battery-grade lithium carbonate from brines, modelling future changes in the energy and chemical reagent sectors and technology optimization. Results reveal wide variation in mitigation potential across projects and highlight trade-offs, notably increased water scarcity impacts (up to 50%) linked to low-carbon heat adoption via heat pumps. Operationalizing scenario-based assessments is key to enabling the practical application of prospective LCA in both academia and industry and driving progress toward decarbonized supply chains.

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5.11.T-03 Material Footprint Method: Beyond Bill of Material to Operationalize Criticality Assessment Methods

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Introduction :

Assessing the criticality of mineral resources has emerged as a pivotal challenge in the European Union, driven by the increasing demand for critical raw materials (CRMs) for electrification and geopolitical uncertainty

regarding their supply. While some traditional approaches to assess how product systems are exposed to criticality focus on the bill of materials (i.e. the quantity of CRM in the final product), this simplification proves inadequate for technologies where consumables, services, or upstream losses play a significant role as they also involve the use of CRMs beyond the final product content. Although the literature identifies the characterization of intermediate flows (IFs) as a potential solution, no CRM-specific framework has yet been operationalized.

The study proposes a first step toward operationalizing a mineral resources use impact assessment method characterizing intermediate flows to assess the life cycle dependency of the product system on CRMs. This framework is illustrated through the case study of the production of 1 kg of hydrogen with an anion exchange membrane electrolyzer stack focusing on the nickel material footprint.

Methods :

1. A matrix Q^* was developed, equivalent to the characterization factor matrix, but tailored for IFs. Q^* is populated with pure CRM values based on stoichiometry and upstream supply chain of each IF characterized. For instance, Q^* (nickel, nickel class 1) contains the amount of nickel per kilogram of nickel class 1 IF.
2. The supply vector is calculated by multiplying the inverse of the technology matrix by the vector of final demand.
3. The material footprint is calculated by multiplying the new matrix Q^* by the supply vector.

Results:

Results obtained using this new framework indicate that producing 1 kg of hydrogen requires 1.62×10^{-3} kg of nickel, with approximately half of this nickel embedded in the energy inputs for electrolysis. Compared to the bill of materials approach, the novel approach yields twice the amount of nickel needed along the value chains, an additional 15% from manufacturing, and a 100% increase from the use-phase electricity.

Conclusion:

This analysis underscores the importance of adopting a life cycle perspective in evaluating the use of critical raw material in product systems. In the context of hydrogen production, the study reveals novel trade-offs between CRMs embedded in electrolyzer stacks and those associated with energy inputs.

Disclaimer/Disclosure:

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5.11.T-04 Development of a Methodology to Include Instrumental Value Loss in Indicators for Abiotic Resources Dissipation in Life Cycle Assessment

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In complement to the concept of depletion, which describes the impact of resource extraction from the biosphere, dissipation focuses on the inaccessibility to abiotic resources once they entered the technosphere. Resources are considered dissipated when they cannot be recovered. The inaccessibility of these resources can be due to economic (not worth recycling) or technological (no technology available) constraints. The risk of supply scarcity, especially for critical raw material, highlights the need to implement strategies towards a circular economy to promote resource efficiency. The economic value dissipation potential (EVDP) method, introduced by Santillán-Saldivar et al., assesses the potential economic value loss due to abiotic resources dissipation by developing a characterization factor for life cycle assessment (LCA). It considers the price and the economic importance at the EU level as a weight factor for the recoverability function specific to the compartment of dissipation. In this work, the recoverability function is modified to incorporate the role of technology maturity, thereby capturing potential recoverability based on technical constraints. Moreover, the resource should only be useful to human activity if it can fulfill certain technical characteristics. A new iteration of the method is proposed, incorporating functionality value loss by using entropy as a proxy for resource purity.

This additional parameter aims to assess the effort required to recover the resource while preserving its purity—and thus its functionality. The effort to recover the resource is calculated, compared to post-primary extraction values, and the result is integrated into the initial EVDP indicator equation. Three metals used in electrical and electronic equipment: gold, copper and germanium, were selected based on their strategic importance and the increasing demand driven by technological advancements to demonstrate the calculations. The results show that recovery efforts are driven by both economic constraints and technological maturity. For critical metals even at a low maturity, its high recovery value can incentivize the optimization of recovery processes. The presentation will explore the potential mitigation of dissipation for strategic metals in printed circuit boards within the French market, with the aim of generalizing this indicator to prospectively assess the benefits of developing new recovery technologies.

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5.11.P Modelling of Environmental Footprints of Critical And/Or Strategic Raw Materials

5.11.P-Th209 Parametric life cycle assessment (LCA) models for metallurgical-grade silicon production using novel processes

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The EU's dependence on critical raw materials (CRMs) is rising as it accelerates the deployment of decarbonization technologies to achieve climate neutrality by 2050. However, the production of these materials often relies on carbon compounds as reductants, especially when hydrogen lacks sufficient potency.

The MECALO (MEtal CARbon LOoping) project aims to develop a groundbreaking concept for producing decarbonized silicon. This approach replaces fossil carbon inputs with carbon black produced from captured and recycled carbon off-gases which is then reused in the reduction of SiO₂. While this concept is still under development, it must be benchmarked against competing reference processes. These reference processes, including conventional processes (with and without carbon capture) and novel modified processes for metallurgical-grade silicon production, have been modelled parametrically using brightway and lca_algebraic. These flexible surrogate models capture the relationships between material and energy flows, enabling their use in the process design phase.

Operationalizing life cycle assessment (LCA) for industrial process design is essential to make sustainability assessments more relevant. By embedding quantitative environmental indicators directly into the design phase. Rather than treating them as post-hoc evaluation, we here enhance the practicality of sustainability methods. To streamline this integration, we developed code that automatically converts parameter, array, and formula dictionaries into lca_algebraic-compatible data. This simplifies the addition and transformation of life cycle inventories without requiring their complete recreation. Our results demonstrate that environmental impacts can be estimated in real time for any configuration of metallurgical-grade silicon production. Variables such as yield, carbon capture rate, carbon input sources, operational region, and reactor setup can be directly translated into a range of life cycle impact assessment outcomes.

Early analyses of carbothermic reactors reveal that the global warming potential is significantly influenced by the national electricity mix across all processes. Prospective LCA indicates that the reference configuration will fall short of the EU's emission reduction targets. In contrast, novel processes, designed for CO₂-neutral silicon production, show potential for achieving lower emissions.

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5.11.P-Th210 Reducing Ecotoxic Impacts in Offshore Operations: Modeling Produced Water CRM Extraction and APG Utilization

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Offshore oil and gas operations in the North Sea generate substantial volumes of produced water and associated petroleum gas (APG), which are traditionally managed through direct discharge and flaring, respectively, contributing to marine pollution and greenhouse gas emissions. This study addresses the challenge of quantifying and mitigating the ecotoxicological impacts of these waste streams on marine ecosystems, including bioaccumulation of contaminants, ocean acidification, and biodiversity loss, while exploring economically viable alternatives that transform liabilities into assets.

We propose an integrated modeling framework to evaluate ecotoxicity before and after implementing two innovative technologies: chemical precipitation for critical raw material (CRM) extraction from produced water and APG redirection to cryptocurrency mining with carbon capture.

The approach couples thermodynamic simulations for optimizing CRM precipitation in high-salinity brines with environmental dispersion models for APG emissions, incorporating metrics such as predicted no-effect concentrations for key contaminants (e.g., lithium, magnesium, boron) and global warming potential equivalents, alongside assessments of pH shifts and plume dispersion under offshore conditions.

Modeling results indicate baseline practices yield high ecotoxicity risks, with produced water discharges exceeding toxicity thresholds for marine biota and APG flaring emitting approximately 4,360 tons of carbon dioxide-equivalent per site annually; post-implementation, CRM extraction reduces contaminant loads by up to 90% and APG mining cuts emissions by over 80%, generating annual revenues of 0.4-0.8 million euros per installation.

These findings demonstrate that the technologies significantly lower ecotoxic burdens without financial strain on operators, achieving net-positive socioeconomic outcomes.

This work advances integrated ecotoxicity assessment in offshore operations by linking waste utilization to sustainability goals, offering scalable applications for regulatory compliance and green energy transitions in the European Union.

5.11.P-Th211 Can mineral production be sustainable? A global ecological footprint assessment of environmental trade-offs

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Minerals and metals are fundamental to global economic development and the transition towards low-carbon technologies. Demand for minerals continues to rise due to population growth, infrastructure expansion, and the rapid deployment of renewable energy systems, batteries, and electric vehicles. However, the environmental pressures associated with mineral production are substantial and increasingly visible. Mining contributes to land transformation, CO₂ emissions, and intensifying competition for freshwater resources—particularly in regions already facing scarcity. These impacts occur simultaneously and often interact, yet most sustainability assessments focus on a single environmental indicator, limiting the ability to understand trade-offs or unintended consequences across impact categories. A more integrated framework is needed to evaluate the environmental sustainability of mineral production holistically. The ecological footprint (EF) provides such a framework by translating diverse environmental pressures into a common unit of biologically productive land area. Although widely used in sustainability research, its application to mining remains limited, with previous studies examining only land use, carbon emissions, or water consumption in isolation. Building on recent advances in quantifying water and land constraints at global scales, this study applies an expanded EF framework that simultaneously accounts for built-up land, carbon absorption land, and water consumption. By integrating these dimensions, we provide a comprehensive global assessment of the environmental burden of mineral production and reveal inherent trade-offs that shape its sustainability.

5.11.P-Th212 Advancing green metal supply-chain decisions with Canadian site-specific LCIs

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The transition toward a net-zero economy requires a rapid expansion in the production of minerals essential for low-carbon technologies. Yet the quality of existing life-cycle inventory (LCI) data remains limited by outdated, incomplete, and non-representative datasets, constraining the ability of Life Cycle Assessment (LCA) to guide sustainable metal supply-chain decisions. This work proposes a methodological framework to generate high-resolution, site-specific LCIs and national market-weighted LCIs, applied to the case of Canada.

The approach builds upon MetalliCan, an open-access, site-level database of Canadian metal production, integrating detailed information on greenhouse gases, pollutants, water use, land occupation, material use, and waste streams. These data are used to construct 50+ site-specific cradle-to-gate LCIs for gold, silver, copper, nickel, and other key metals, which are directly implemented in Brightway. A modular LCI modeling framework enables dynamic assembly of mining, beneficiation, and refining stages, while parameterizing variability in ore type, ore grade, deposit type, and mining method. Data gaps are addressed using parametric relationships and the lca-algebraic tool, enabling transparent quantification of the influence of critical parameters on environmental performance.

LCAs are performed using Impact World+ 2.1, a globally regionalized LCIA method. The resulting site-specific LCIs are aggregated into market-weighted Canadian LCIs, representing average metal consumption in Canada. In a second step, these LCIs are used to compare Canada's environmental performance against production in other world regions using literature-based inventories. Several scenarios are assessed, including: (i) mining and beneficiation in Canada with refining abroad; (ii) full Canadian supply-chain control (mining, beneficiation, and refining in Canada); and (iii) scaling impacts under existing and potential future production capacities. The framework also evaluates sustainability implications of future metal demand required to achieve carbon neutrality.

Overall, this work demonstrates the value of high-quality, site-specific and parametric LCIs for characterizing the environmental performance of metal supply chains, identifying opportunities for strategic relocalization, and informing evidence-based mineral policy in Canada.

5.11.P-Th213 Material Footprint of Japan based on the 2020 Input-Output Analysis and Development of Circularity Indicators

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Policies promoting the circular economy have advanced rapidly worldwide. In the European Union, the proposed Circular Economy Act aims to strengthen markets for secondary raw materials and integrate circularity into industrial competitiveness and clean-technology strategies. In Japan, the Fifth Fundamental Plan for a Sound Material-Cycle Society, adopted in August 2024, positions resource circulation as a national priority with concrete directions for the next five years.

Despite this policy momentum, academic and practical discussions still lack a unified framework for assessing circularity. Major challenges include (i) substantial variation in how circularity is defined and measured across studies and (ii) insufficient alignment between proliferating indicator sets and the needs of circular business models or resource-circulation policies. Although international standardization is progressing, with ISO 59020 providing a framework for circularity assessment and clarifying principles for boundary setting and indicator selection, it remains limited to a measurement framework and does not prescribe harmonized indicators for quantifying national resource use. Consequently, complementary and empirically grounded quantitative indicators are still needed for policy evaluation.

To address this gap, this study focuses on the Material Footprint (MF) as a comprehensive indicator of national-level resource use. MF, the official SDG 12.2.1 indicator, attributes global primary material extractions to a country's domestic final demand across four resource categories—biomass, fossil fuels, metal ores, and non-metallic minerals—capturing upstream extraction embodied in trade and enabling international comparability.

We integrated the 2020 Input–Output Table with raw material equivalent (RME) coefficients to estimate the material extractions induced by Japan's domestic consumption of a wide range of products and services. This enabled quantification of upstream resource use and derivation of sector-level resource intensity indicators. We also examined the consistency between MF and key components of circularity indicators referenced in ISO 59020, evaluating MF's potential as a foundational indicator for circularity assessment.

The results provide a quantitative basis for evaluating resource use at both product and sector levels, contributing to the development of more robust indicators for advancing the circular economy.

5.11.P-Th214 Environmental Case for Formalising Artisanal Cobalt Mining in the Democratic Republic of the Congo: Life Cycle Assessment for Europe's Green Transition

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Cobalt is indispensable for the EU's green and digital transition, yet its dominant supply from the Democratic Republic of the Congo (DRC) poses severe sustainability challenges. While the social risks of artisanal and small-scale mining (ASM) (such as child labour) have spurred calls for formalisation, the environmental trade-offs between different mining methods remain poorly quantified. A critical barrier is the lack of region-specific inventory data for DRC that includes ASM, undermining robust environmental footprints and hindering evidence-based policy for securing critical raw materials.

To bridge this gap, we conducted a life cycle assessment (LCA) of three cobalt mining systems: 1) Large-scale mining (LSM), 2) LSM with integrated formal ASM, and 3) LSM with informal ASM. The LCA models were built on life cycle inventories (LCI) that accurately reflects DRC-specific conditions, including formal and informal ASM. We compared the environmental performance of these systems under a baseline and alternative scenarios, analysing variables such as tailings management, transport logistics, and the national electricity mix. Our analysis demonstrates that formalising and integrating ASM with LSM can significantly mitigate the adverse environmental impacts of informal ASM, bringing its performance close to that of regulated industrial mining. Contribution analysis identifies explosive blasting, milling machinery-driven steel depletion, mine construction, and diesel-based transport as main hotspot processes. The environmental impacts across 18 categories of ReCiPe 2016 midpoint (H) are primarily driven by energy production for material manufacturing and transport operations, as well as contaminants from operational supply chains of the hotspot processes. This study provides the first quantifiable environmental rationale that formalising ASM cobalt in the DRC not only addresses pressing social concerns but also offers environmental benefits. We advance the field by introducing a critically needed LCI dataset that includes ASM and DRC-specific parameters, directly addressing methodological gaps in critical raw materials assessments. Our transparent, scenario-based approach offers actionable insights for EU policymakers and industries, supporting the development of secure, responsible, and scientifically informed raw material strategies.

5.11.P-Th215 Methodological Barriers in Life Cycle Assessment of Advanced Materials: Insights from the MACRAMÉ Project

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The increasing use of advanced materials such as graphene oxide, few-layer graphene, carbon nanotubes and polymer-based nanostructures raises important questions about their environmental sustainability. Life Cycle Assessment (LCA) offers a well-established framework for evaluating impacts, yet current LCA methodologies are not fully adapted to the specific behaviours, release mechanisms and toxicity of nanomaterials.

A central challenge is the lack of nanoform-specific data, emission factors and toxicity characterisation factors in widely used databases and Life Cycle Impact Assessment Analysis (LCIA) methods. This hinders robust impact quantification and limits the implementation of Safe and Sustainable by Design (SSbD) for emerging materials.

To address these limitations, the MACRAMÉ project has developed detailed Life Cycle Inventory datasets for four representative nanomaterials across varying Technology Readiness Levels. Missing datasets in ecoinvent v3.11 were reconstructed using literature and patent-based synthesis routes, proxy substances adjusted for molecular weight, and expert-derived assumptions. Material Flow Analysis data were integrated to approximate releases in the absence of empirical nanoform measurements.

The results highlight persistent methodological barriers: dependence on proxies, insufficient release quantification, and the lack of characterisation factors for human toxicity and ecotoxicity. These limitations lead to considerable uncertainties in impact assessment.

The MACRAMÉ approach provides a structured pathway towards improving LCA methodologies for nanomaterials and contributes essential foundations for future SSbD-oriented evaluation frameworks.

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5.11.P-Th216 Modelling Environmental Footprint of Neodymium and Dysprosium in Nd-Fe-B Magnets and Benefits of Reducing Dy Content

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Neodymium (Nd) and Dysprosium (Dy) are critical raw materials essential for the production of high-performance Nd-Fe-B (Neodymium-Iron-Boron) permanent magnets used in green technologies, such as electric vehicles and wind turbines. However, they are associated with geopolitical supply risks due to their concentrated production in China; their extraction also causes considerable environmental damage and social risks, particularly in the unregulated mining regions of Myanmar. As the global demand for these magnets increases, several studies have examined the technical feasibility of reducing their Dy content, while maintaining magnet performance, through material-efficiency techniques, but none have specifically assessed the environmental implications of such a reduction.

This study evaluates the environmental impacts of varying Dy content (1 to 8 wt.% Dy) in sintered Nd-Fe-B magnets using life cycle assessment. We modelled all processes, from raw material extraction – including Nd from the Bayan Obo mine in China and Dy from ion adsorption clay deposits in Myanmar – to the production of a shaped magnet. Six magnet compositions were assessed using the 16 impact categories of the Environmental Footprint method. Contribution analyses were performed to quantify the relative role of Nd and Dy in the overall magnet impacts and to identify hotspot processes.

Results show that, despite representing only a small fraction of the magnet composition, Dy has a disproportionate contribution to environmental impacts, particularly freshwater ecotoxicity, terrestrial and marine eutrophication and land use. Reducing its content, systematically reduces impacts in all categories, even despite increased Nd content. Each 1 wt.% reduction in Dy content prevents the leaching of 220 kg of ore and avoids 4.7 kg of CO₂-eq emissions per kilogram of Nd-Fe-B magnet. For low-Dy content magnets (1 wt.%), Nd oxide production is the most significant contributor to all impact categories, including over 80% of ozone depletion and human carcinogenic toxicity.

By modelling the entire supply chain, the results demonstrate that reducing Dy content through material efficiency is an effective strategy that limits depletion of critical raw materials and significantly reduces environmental footprint of Nd-Fe-B magnets.

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5.11.P-Th217 How Data and Modeling Choices in Critical Raw Material Supply Chains Shape the Environmental Footprint of Electric Vehicles

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The transition to global net-zero greenhouse gas emissions relies on electric vehicles (EVs), whose environmental performance is strongly influenced by the supply chain of critical raw materials such as lithium, cobalt, graphite, and neodymium. Lithium-ion batteries and Nd-Fe-B permanent magnets rely on these critical raw materials. Life Cycle Assessment (LCA) is central to evaluating the potential environmental impacts of these components; however, further methodological harmonisation is required to achieve comparability and compliance with emerging European regulations (e.g., the Battery Regulation, the Critical Raw Materials Act, and the Corporate Sustainability Reporting Directive). This study applies regulation-aware LCA guidelines developed within the MaDiTraCe project to assess a product system comprising an NMC811 battery pack and Nd-Fe-B magnets.

The analysis adopts a cradle-to-gate-plus-grave perspective, with a basket functional unit, encompassing the functions provided by the battery and rotor magnets. Datasets for Li, Co, Nd, graphite, magnets and battery were sourced from peer-reviewed literature and adapted for guideline compliance, compatibility with ecoinvent v3.10.1 and reproducibility in open-source software. The impact assessment followed the Environmental Footprint LCIA method v3.1. We explored alternative modelling decisions, including recycling allocation and sourcing scenarios, and their effects on the environmental profile of the EV components.

The battery supply chain accounts for most of the environmental burden of the product system, while the magnets contribute less than 7% in every impact category. Anode and cathode manufacture and cell assembly dominate impacts in 13 of 16 categories (28%-87%), while cathode raw material acquisition drives Ionising

radiation (56%), Particulate matter (34%) and Water use (68%). Modelling decisions significantly affect results. Allocation by substitution in recycling scenarios offsets burdens, leading to apparent benefits in acidification. Commodity sourcing (e.g., Li from brine vs. spodumene) alters climate change impacts by up to 20%. Harmonised LCA practices improve comparability and transparency in assessing EV components, supporting compliance with EU regulations and informed decision-making. Results inform hotspot-based data collection efforts and demonstrate the influence of modelling choices on outcomes.

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5.11.P-Th218 New Life Cycle Inventories of Rare Earth production from Brazilian ion-adsorption clays and Australian heavy mineral sands based on mining project Feasibility Studies

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Rare earth (RE) elements are considered critical raw materials and their demand is projected to increase significantly over the next decades, especially in the applications for the energy transition, digitization and defence. In response to the increased global demand and the high reliance on China in RE mining and refining, many new mining projects are pursued outside China. Two types of deposits are gaining importance for their ease of mining and reduced complexity compared to hard-rock carbonatite mining; ion-adsorption clay (IAC) and heavy mineral sand deposits. However, there are no LCA studies for the production of RE from heavy mineral sands, while for IACs life cycle inventories (LCIs) are only available for the in-situ leaching method, but not for open pit mining with a central leaching plant like proposed for 2 largescale projects in Brazil. To close this gap, 2 new LCIs for RE production from IACs via open pit mining and leaching at a central processing facility, and from heavy mineral sand deposits are developed. These LCIs will be based on a review of existing LCIs for RE production as well as publicly available data from feasibility studies. For the IACs feasibility studies of the Colossus and Caldeira project in Brazil will be used, and for the heavy mineral sands studies of the Donald, Wimmera, and Goschen projects in Victoria, Australia. To also make the LCI results applicable to similar mining projects, a special emphasis will be placed on the relationship between ore characteristics and the magnitude of flows. This will help to better understand the environmental impacts of the global RE industry in the future and identify advantages and drawbacks of different RE production routes.

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5.11.P-Th219 Evaluating End-of-Life Recycling Pathways of Battery Electric Vehicles: A Life Cycle Assessment Approach

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The rapid rise of battery electric vehicles (BEVs) has introduced new environmental and resource-related challenges, particularly as these vehicles approach end-of-life (EoL). Unlike conventional internal combustion engine vehicles, BEVs contain complex components enriched with critical raw materials, making their end-of-life treatment both an opportunity and a challenge for recycling systems. As Europe accelerates its transition toward electrified mobility and also on the release of the European directive for End of life of vehicles, understanding how current recycling processes perform becomes increasingly important for ensuring material circularity and minimizing environmental impacts. This study addresses the central question of whether existing EoL recycling strategies for key BEV components are environmentally efficient and capable of supporting future circular economy goals. To investigate this, the research focuses on four major subsystems traction batteries, electric motors, copper wiring harnesses, and automotive shredder residue (ASR) chosen for their high concentrations of valuable and critical materials. The challenge is approached through a Life Cycle Assessment (LCA) framework, which evaluates the environmental burdens associated with current recycling processes. Using established LCA methodology, the study aims to identify hotspots within recycling chains, and compares the relative impacts of the selected components.

The results show that EoL of traction batteries exhibit the highest GWP, driven by energy-intensive processing steps and the presence of lithium, cobalt, nickel, and other critical materials. The remaining components demonstrate lower GWP values; however, the remaining components presents significant uncertainty due to its heterogeneous composition and inconsistent recovery routes, despite its comparatively low modeled GWP in SimaPro.

The study concludes that improvements in material separation, targeted recycling technologies, and more comprehensive datasets are essential to enhance the environmental performance of BEV recycling. These insights are highly relevant for policymakers, industry stakeholders, and researchers, as they support the development of more efficient EoL systems and contribute to advancing circularity and sustainability within the rapidly evolving automotive sector.

5.11.P-Th220 LCA Analysis of Forged Steel Production: Product Assessment and Optimization Strategies for Improved Environmental Performance

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The European Green Deal and the Fit for 55 legislative package aim to reduce greenhouse gas emissions across the European Union and achieve climate neutrality by 2050. In this context, the LIGHTFORGE project aims to develop low-density Fe-Mn-Al-C steels suitable for industrial forging to reduce the weight of vehicles. This study assesses the environmental impacts associated with the production of a forged component (drop arm) manufactured from conventional 42CrMo4 steel, using the life cycle assessment methodology. A cradle-to-gate system boundary was used, with a functional unit of 1 ton of finished product. The data used in the study includes primary information provided by the companies involved in the project and secondary data sets from database such as Ecoinvent. The assessment was carried out using SimaPro software and the ReCiPe impact assessment method.

The results show that over 60% of the impacts in the categories analyzed come from billet production processes. For this first part of the process, the steelmaking phase is the most critical due to the use of raw materials such as ferromanganese, ferromolybdenum, ferrochromium, and silicon. Hot rolling has been identified as the main critical phase for the categories of water consumption, fossil resource scarcity, and ionizing radiation. The environmental impacts associated with forging the drop arm are principally caused by the consumption of electricity.

A sensitivity analysis examined two scenarios: replacement of the Spanish electricity mix with photovoltaic energy and changing from road to rail transport for billets. Increasing the share of renewable electricity leads to significant reductions in several impact categories, while shifting transport modes produces relatively minor benefits.

The study identifies billet production, particularly the steelmaking phase, as the main critical point and suggests optimizing the use and sourcing of materials and the use of renewable electricity to reduce environmental impact. Future work will compare these results with drop arm manufactured from low-density Fe-Mn-Al-C steels and extend the assessment to include the use and end of life phases.

5.11.P-Th221 Can we Save Materials by Adding Materials? Exploring the Advanced BMS Paradox

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Batteries are a cornerstone technology for the energy transition, and substantial resources are being mobilized to rapidly expand the battery production capacity in Europe. However, this expected growth is linked to a critical challenge. Batteries depend on several critical raw materials, including natural graphite, cobalt, silicon metal, lithium and manganese, and the availability of these materials is limited. A key question for the coming years is how Europe can expand battery capacity while remaining within material supply boundaries. Extending battery lifetime offers one promising pathway to limit the raw-material demand associated with increased battery capacity. However, this introduces a fundamental tension, because lifetime extension typically relies on additional sensors, electronic components and energy use, all of which also require critical raw materials. This work evaluates this trade-off by assessing the environmental impacts of an advanced battery management system (BMS) developed in the NEMO project. Through improved temperature, voltage, current and cell-

impedance sensing, as well as advanced modelling, machine learning and cloud-based data analysis, the project aims to extend battery lifetime and increase safety. By performing a comparative life cycle assessment and life cycle costing of an NMC523 battery with a benchmark BMS and two advanced BMS concepts, this study examines whether the combined benefits of added lifetime and safety outweigh the environmental burdens related to the additional hardware. A central methodological challenge that will be explored is how to explicitly include the added function of increased safety relative to a benchmark BMS in the functional unit, for example by estimating changes in failure probabilities and ageing. Additionally, detailed life cycle inventories (LCIs) are created for a benchmark system based on the open source foxBMS 2 platform and for two advanced systems that incorporate data from the NEMO project. These inventories represent a significant improvement in the quality of available BMS data compared with the LCIs currently provided in ecoinvent. The work also develops two prospective scenarios to explore how different future conditions related to raw material availability may influence the comparative results. At the conference, an overview of the methodological approach and the main challenges encountered will be presented, together with preliminary results if they are available.

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5.11.P-Th222 Operationalizing the Characterization of Potential Impacts of Dissipation: an Exploration through the Ecoinvent Database

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International recommendations from UNEP-SETAC Life Cycle Initiative points towards the characterization of mineral and fossil resource loss, the so-called dissipative flows. However, life cycle inventory (LCI) databases do not include resource dissipative flows but only extraction flows. Many life cycle impact assessment (LCIA) methods characterizing dissipation have been proposed but are still not operationalized due to missing dissipative flows in LCI databases. The objective is to propose a series of proxy allowing to operationalize those LCIA methods despite the discrepancy between inventory flows and characterized flows. This operationalization is illustrated with the ACP and RESEDA methods. To reach that general objective, three subobjectives are defined: develop an operational proxy classification method, apply it to characterize the entire ecoinvent database with ACP and RESEDA methods and explore the contribution of the foreground and background of processes to the resources services loss and discuss the limits of the proxy classification proposed. To address the first subobjective, we classify emissions of metals to the environment as dissipative flows, either as pure element or embedded in chemicals (aluminum in Aluminium hydroxide). Metal flows inputs to tailings and landfills are dissipative, however are intermediate flows in LCI databases. The proxy chosen for those intermediate flows is the output long-term emission to the environment. Regarding fossil resources, we identify two dissipation pathways: combustion and emissions of embedded fossil carbon in chemicals and plastics to the environment. To address the second subobjective, we implemented the newly developed characterization model into brightway2 with ecoinvent 3.11 cutoff version. The potential ACP impact score is mostly coming from background processes, revealing the importance of operationalization at the LCI database level. We address the third subobjective by adding missing dissipative flows to some ecoinvent processes, identified through study of mass imbalance between inputs and outputs and experts' knowledge, to then study ecoinvent processes potential impact change relatively to the original ecoinvent database, using the ACP indicator. The limits of proposed proxies are explored in depth to provide a full picture of impact results uncertainties. We demonstrate the operationalization of both ACP and RESEDA methods of metallic and fossil resources dissipative flows.

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5.11.P-Th223 Future Welfare Loss Method

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Sub-soil resources such as minerals and fossil fuels are exhaustible, and their accelerated use raises concerns about the burden imposed on future generations. Existing approaches either assume that market prices already internalise all social costs, as is common in Cost–Benefit Analysis (CBA) or rely on long-term and highly uncertain physical forecasts of ore grades, technologies and extraction costs, as in Life Cycle Assessment (LCA).

We performed a study which addresses the challenge of valuing the social cost of depleting sub-soil resources in a way that is theoretically sound, empirically feasible and directly usable in CBA and LCA. We propose a market-price-based valuation method that exploits the information embedded in current resource prices while correcting for the divergence between private and social discount rates. Using Hotelling's rule, we model the time path of the net resource price (market price - extraction cost) and show how a higher market discount rate, compared to a lower social discount rate, leads to underpricing of resource depletion.

Empirically, we use five-year trend prices for a wide range of mineral and fossil resources, extraction costs (mainly from Eurostat data), and assumptions on market and social discount rates, with alternative scenarios for time to depletion and declining rates. For each resource, we compute a price correction factor from the ratio of integrals of market and social discount factors, which, multiplied by the scarcity rent, yields the externality cost of depletion. Results indicate substantial positive social costs, often amounting to several tenths of the current price and reaching or exceeding the market price for high-rent, relatively scarce resources such as crude petroleum and copper.

The proposed factors provide a practical set of social cost values for sub-soil resources that are consistent with economic theory yet avoid speculative long-term scenario modelling. These factors can serve as monetary characterisation factors for abiotic resource depletion in LCA and as correction terms in CBA, thereby improving the treatment of resource use in environmental decision-making, supporting more sustainable choices on extraction, recycling and technology development.

We are proud to add that our method is being recommended by GLAM (United Nations Environmental program).

Disclaimer/Disclosure:

The study has been published and can be found here: <https://www.mdpi.com/2079-9276/8/1/19>

Recommendation of the method by GLAM: https://www.lifecycleinitiative.org/wp-content/uploads/2024/10/GLAM-2024_Summary-NR.pdf

Track 6: Environmental Policy, Risk Management, and Science Communication

6.01.P An Early Warning System for Chemicals Enables to Identify Emerging Risks

6.01.P-Th224 European Stakeholder Perspectives on Early Warning Systems (EWS) for Chemicals – Insights From a Survey

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An Early Warning System (EWS) for chemicals is being developed within the Horizon Europe Partnership for the Assessment of Risks from Chemicals (PARC) to address the growing number of substances and their potential risks. As previous consultations with stakeholders on EWS in general have been limited, this study focused on investigating expectations and familiarity of European stakeholders, as a basis to improve the PARC EWS. A stakeholder survey was developed, validated and disseminated across European and national organisations. The categorical answers were evaluated using Fisher's exact test to examine potential differences among types of organisation (e.g. governmental agencies and academia). In addition, a Stakeholder Interaction Forum (SIF) was organised to discuss survey results, stakeholder expectations, as well as EWS communication strategies and data needs.

In total, 61 individuals responded to the survey, mainly from government agencies (n=25), research institutes (n=16) and academia (n=11). Most participants considered an EWS "very" or "somehow" important, with significant variation among organisation types ($p < 0.05$). Awareness and understanding of existing EWS differed widely, indicating a need for clear terminology. 41 % reported having previously considered outputs from EWS or similar systems. The number of respondents choosing "conducting further research" as one of the reactions to an early warning signal varied significantly among the organisation types. Answers also varied regarding preferences for EWS communication channels and the EWS inputs that were considered valuable for signal evaluation. Most respondents (79 %) reported a willingness to receive potentially uncertain alerts. The desired EWS update frequency was scattered in the survey, while yearly updates with the option for rapid alerts in urgent cases was judged most feasible during the SIF.

The combined survey and SIF outcomes provide direct guidance for further development of the EWS. The investigation indicates that an EWS architecture shall become flexible to accommodate differing needs, that moderate uncertainty in signals is deemed acceptable, and that annual EWS updates, as well as an event-driven fast-track option, are desired. We anticipate a continuously strong stakeholder engagement in future planned activities, which will further inform the development of EWS on EU and European national level.

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6.01.P-Th225 Archived Suspended Particulate Matter as an Early-Warning Tool: 18-Year of Non-Target Screening Identifies Emerging Contaminants and Regulatory Success

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Early detection of chemical trends is critical for proactive environmental protection. This study demonstrates the potential of archived suspended particulate matter (SPM) as an early-warning tool for contaminants of emerging concern (CECs). SPM collected between 2005 and 2022 from two Rhine River sites (Weil am Rhein and Koblenz) within the German Environmental Specimen Bank was analysed using liquid chromatography–high-resolution mass spectrometry (LC-HRMS) with suspect and non-target screening. Structural annotation, supported by retention-time indices and multiple MS/MS evidence sources (mzCloud, FISh, CFM-ID), yielded 332 compounds across confidence levels (Level 2.1: 3%, 2.2: 6%, 3.1: 53%, 3.2: 38%). Trend analysis showed that 25% of detected chemicals increased significantly over time, with stronger increases at Koblenz (29%) than at Weil (18%). Increasing compounds included pharmaceuticals, industrial chemicals, surfactants and biocides, whereas 13–14% of features declined, reflecting regulatory measures and improved wastewater treatment.

Notable decreases included PFOS, diazinon, carbamazepine and climbazole. ECOSAR-based hazard prediction indicated that nearly half of annotated structures (47%) pose high acute toxicity ($LC_{50}/EC_{50} > 1 \text{ mg/L}$) to aquatic organisms. By exploiting archived SPM for retrospective and trend-based analysis, this approach enables early identification of emerging contamination, supports evaluation of regulatory success and provides actionable watch lists for targeted monitoring and risk management. Complementary effect-based analysis (EBA) and GC-HRMS investigations were conducted separately to further broaden the chemical and toxicological scope of this early-warning framework.

6.01.P-Th226 Early detection of potential human and ecological risks of Critical or Strategic Raw Materials in water

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This presentation will show work that started as a the outcome of a national early warning approach for chemicals that potentially pose risks in waters in the near future. Here, we assessed Critical or Strategic Raw Materials (CRM, SRM) as a sharp increase is expected in the use of these compounds in the next 20 years, which could ultimately lead to increased concentrations in surface water and drinking water. As a start, we identified that seven critical materials that warrant extra attention due to their environmental hazards and limited policy focus: cerium, dysprosium, gadolinium, germanium, neodymium, palladium and platinum. Monitoring of these substances is still limited, making it difficult to determine their origin and trends. In this study, we compiled available monitoring and (eco)toxicological data to conduct a preliminary assessment of the occurrence of these metals in Dutch surface waters and their associated risks. Monitoring data for surface water, wastewater and drinking water were retrieved from national databases, and the collected (eco)toxicological data include data relevant to organisms in surface water and to human exposure via drinking water. Surface water monitoring databases contained data for a subset of these five of seven metals (cerium, dysprosium, gadolinium, neodymium and platinum). All seven metals were all investigated in wastewater, but the concentrations were often below the detection limits exceeding than those used for surface water. Overall, concentrations in wastewater and surface water were generally comparable. Ecotoxicological data for freshwater organisms are available for all seven metals. We compared the average concentrations in surface water and wastewater to the available ecotoxicological standards to assess whether exceedances occur. Some of the compounds, including cerium, gadolinium and neodymium, have been measured in drinking water sources (before treatment), yet human toxicological data were only available for cerium and gadolinium. These findings show important gaps in both monitoring and toxicity data. Our assessment provides water authorities and utilities with early insight into emerging CRM/SRM pressures and supports future environmental and human health risk evaluations.

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6.01.P-Th227 Chemicals In Energy-Transition Technologies: Cross-Sector Inventory And Development Of A New Risk Indicator

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The rapid deployment of low-carbon technologies is increasing the variety of chemicals used in energy systems. However, many of these substances have not yet been adequately characterised in terms of their environmental behaviour and hazards. Anticipating these risks is essential to support strategic decisions in energy decarbonisation pathways.

This work aims to develop a harmonised, cross-technology inventory covering batteries, hydrogen systems, photovoltaics, nuclear applications, wind power and CO₂ capture processes. The chemicals and materials were identified through expert workshops, technical documentation, safety data sheets and scientific literature. A multi-source approach combining industrial reports, quantitative calculations and expert judgement was applied to estimate quantities used. The substances were classified into key hazard groups, including carcinogenic, mutagenic and reprotoxic compounds; heavy metals; persistent and mobile chemicals; fluorinated polymers; solvents; and engineered nanomaterials.

More than 430 chemicals were identified across the studied technologies. The highest prevalence of priority substances was found in batteries, hydrogen systems and photovoltaic technologies, including persistent

fluorinated salts and polymers, toxic metals and nanomaterials.

A set of qualitative and quantitative risk indicators was developed to conduct cross-sector and cross-technology risk comparisons, combining hazard information with exposure parameters. This approach ensures the integration of both well-characterised substances and those for which data are limited.

Overall, this study is one of the first to provide an integrated assessment of chemical use across energy-transition systems. It offers a scalable tool for identifying priority substances to support future environmental policy and risk-management decisions.

6.01.P-Th228 A Network and Cheminformatics Approach to Reveal Human Protein Targets of Endocrine-Disrupting Chemicals

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Endocrine-disrupting chemicals pose a persistent challenge for chemical risk assessment, as their effects often emerge from diverse and sometimes unexpected interactions with human biological systems. Anticipating such risks requires tools capable of detecting early signals before adverse effects become evident. To contribute to this need, we developed ED-Net, a network-based framework designed to identify potential human molecular targets relevant to endocrine disruption.

To develop ED-Net, we adapted an established approach that builds networks of proteins responding to similar chemical influences. In simple terms, when several endocrine disruptors affect the same proteins, these proteins are grouped together because they likely share related biological roles. Each affected protein becomes a node in the network, and we connect two proteins only when several endocrine disruptors act on both of them, indicating a meaningful shared response. This produces a clear and reliable map of protein relationships in the context of endocrine disruption.

Complementing these associations, ED-Net integrates structural fingerprint analysis to reveal molecular similarities between chemicals and support the identification of plausible protein targets. The model allows users to explore known proteins, examine putative targets, and access descriptive information for each protein or chemical, including their classification and links to external resources such as UniProt or PubChem. ED-Net will be available as a web platform.

Initial results indicate that ED-Net can uncover biologically relevant targets for chemicals with limited toxicological data and highlight new associations consistent with endocrine-related pathways. By combining network science and cheminformatics, ED-Net provides a powerful tool to enhance early warning systems for endocrine disruptors and support prioritisation efforts in regulatory toxicology.

Disclaimer/Disclosure:

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6.01.P-Th229 Embedding Health in Climate Adaptation: Research Directions for Drinking Water Systems

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Climate change is reshaping water systems globally. In specific cases, it has a profound consequences for human health. Rising temperatures, changing precipitation patterns, and more frequent extreme weather events are disrupting both the quality and availability of drinking water. The Netherlands, known for its advanced water management, is also vulnerable to climate change induced extreme events, such as, flooding, drought, and salinization. These events pose immediate challenges to public health and the resilience of water infrastructure. Our study examines the climate–water–health nexus with a focus on the Dutch water system. We assessed key health risks, policy integration, knowledge gaps and research priorities. Through a comprehensive literature review, policy analysis, and interview with the experts in the field of water management, public health, climate change, and governance, we identified direct health risks, including increased microbial and chemical contamination, as well as, broader impacts, such as food insecurity and mental health concerns. While water

governance in the Netherlands aligns closely with EU climate objectives, gaps persist in policy implementation and the integration of health considerations. Critical research needs include (near) real-time monitoring, improved understanding of microbial and chemical dynamics under climate stress, and stronger integration between science, governance, and public engagement. To address these challenges, we propose a research roadmap that prioritizes the development of health risk indicators, innovation in monitoring and data systems, designing and testing of climate-resilient (drinking) water infrastructure, and embedding research into policy frameworks. Our findings emphasise the need for integrated, evidence-based adaptation strategies to safeguard public health and strengthen the resilience of Dutch drinking water systems in the face of accelerating climate change.

6.02 Bringing “Design” back to SSbD: Designing Molecular Structures for Safety and Sustainability

6.02.T-01 Designing What to Assess in SSbD: A Pathway Builder Integrating Governance Mapping and Morphological Analysis

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The European Commission’s Safe-and-Sustainable-by-Design (SSbD) framework distinguishes a (re)design phase from the assessment. In practice, however, SSbD efforts – including those emerging in healthcare – mostly start from a short list of options and focus on evaluating them, rather than structuring how options are generated and governed. This can lead to narrowly framed choices and “forever chemical” lock-ins, as seen for per- and polyfluoroalkyl substances (PFAS).

This work addresses two related challenges: (i) the lack of an explicit meso-level pathway builder that links macro-level problem framing to micro-level design options across the life cycle, and (ii) the absence of clear responsibility mapping for who can change which options. A governance-aware, morphological pathway builder is presented, integrating (i) a macro–meso–micro framing of the design problem, (ii) Responsibility-Accountable-Consulted-Informed (RACI) governance mapping of stakeholder roles along the value chain, and (iii) General Morphological Analysis (GMA) with Cross-Consistency Assessment (CCA) to explore and systematically prune a constrained morphological design space.

The methodology is implemented as a spreadsheet-based prototype and illustrated for single-use injection devices and associated packaging, shaped within the EU Innovative Health Initiative (IHI) ENKORE project. Even conservative parameterisation yields many “device-packaging-logistics-use-EoL” combinations. Applying consistency checks will further remove infeasible and non-compliant configurations, delivering a manageable portfolio of governed, assessment-ready pathways that respect sterility, regulatory and logistical constraints while making explicit which stakeholders must be involved in what implementation.

Rather than selecting “best” designs, the proposed pathway builder structures transparent design pathways that can subsequently be evaluated with life cycle, risk or other assessment workflows. It offers a concrete way to “bring back the design” into SSbD practice and to support safer, more circular, and better-governed innovation pathways for healthcare devices, reducing the risk of future PFAS-like crises.

6.02.T-02 Interactive Generative Modelling for Inverse Design of Safe and Sustainable Antioxidants

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Ensuring the safety of novel chemicals requires proactive strategies that consider environmental and health hazards already at the molecular design stage. Phenolic antioxidants are widely used and increasingly considered contaminants of emerging concern, exemplifying the need for molecular (re)design that maintains function while reducing risk. This work addresses this challenge by developing an interactive generative modelling framework for the de novo design of safer phenolic antioxidants aligned with Safe and Sustainable by Design (SSbD) principles.

We employ a genetic algorithm to generate and evolve molecular structures while optimizing four key criteria: synthesizability, antioxidant activity, biodegradability, and (eco)toxicity. Synthesizability is assessed with heuristics; antioxidant activity is approximated via O–H bond dissociation energies and ionization potentials; ready biodegradability is predicted using a machine-learning model developed by Körner et al. (Environ Sci:

Proc Imp 2024); and baseline (eco)toxicity is estimated through log Kow. A human-in-the-loop interface enables domain experts to provide real-time feedback guiding the optimization. The user can dynamically adjust objective weights, remove undesirable structural motifs or add promising structures, integrating intuition that is otherwise difficult to capture.

The genetic algorithm efficiently identifies a diverse set of promising phenolic structures with improved predicted profiles for performance and safety. Molecular similarity analysis confirms high structural diversity among the generated molecules, which supports a broad exploration of independent structural strategies. Comparison with a curated set of ~500 antioxidants in current use shows that early generations of molecules generated by the genetic algorithm rediscover known molecules, while later generations explore increasingly novel chemical space.

This work demonstrates how generative modelling, expert interaction, and predictive hazard assessment can be integrated into a practical SSbD-oriented design workflow. It is embedded in an interdisciplinary project in which our project partners are developing experimental high-throughput methods for assessing persistence, toxicity, and degradation-product hazards. The project lays the foundation for a closed-loop design approach coupling high-throughput experimentation and generative modelling via active learning to accelerate the development of safer functional chemicals.

6.02.T-03 An Integrated Early-Stage SSbD Workflow Using AI/AOP-Based NGRA and Streamlined LCA for Chemical Substitution

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Early-stage Safe and Sustainable by Design (SSbD) decision-making requires simultaneous consideration of chemical safety and environmental sustainability. While AI-driven predictive toxicology and Adverse Outcome Pathway (AOP)-based Next-Generation Risk Assessment (NGRA) have advanced mechanistic hazard evaluation, they are rarely integrated with life-cycle sustainability metrics. This separation can lead to unbalanced or misleading substitution choices, particularly in consumer product applications. In this study, we propose a practical early-stage SSbD workflow that combines (1) AI/AOP-based NGRA for rapid identification of mechanistic hazard hotspots, and (2) a streamlined cradle-to-gate Life Cycle Assessment (LCA) to assess environmental performance with minimal data requirements. The two assessment streams are qualitatively linked through a simple risk assessment (RA)–LCA matrix to highlight alignment, conflicts, and potential trade-offs. To illustrate feasibility, the workflow is applied to a representative set of alternative chemicals commonly used in consumer products (e.g., plasticizer or bisphenol analogues). The results demonstrate that integrating AI-based hazard prediction with simplified LCA provides balanced and actionable early SSbD insights, enabling prioritization of safer and more sustainable alternatives before detailed testing or full LCA is conducted. This framework offers a lightweight, scalable approach that can support rapid design iterations and early innovation in chemical substitution.

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6.02.T-04 Integrated Methodology and Decision Support System for Safe and Sustainable Substitution

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To enable the practical implementation of the Safe and Sustainable by Design (SSbD) approach in developing safer and more sustainable alternatives to replace substances of concern, the HORIZON project PLANETS is developing an integrated methodology and a Decision Support System (DSS). The integrated methodology enables assessments across all relevant SSbD dimensions: human and environmental safety, environmental, economic, and social sustainability and functional performance. It supports decision-making and the identification of optimal and robust design choices. Unlike fixed aggregation methods, the approach is flexible and tailored to data availability, quality, and stakeholder needs.

The DSS builds on the JRC SSbD framework and extends into a digitally operational tool incorporating diverse assessment methods, including in silico modelling, experimental assays, life cycle assessment, life cycle costing,

performance testing and social impact assessment. The DSS provides an interactive environment for selecting relevant SSbD aspects, integrating assessment results, applying optimization and multiple-criteria decision analysis, and visualizing trade-offs using tools like spider charts, Pareto fronts, and heatmaps. Three industrial real-life cases—surfactants, flame retardants, and plasticizers—are employed to test and optimise the integrated methodology and DSS. Initial results show successful integration of heterogeneous data, improved transparency in decision making, and support for exploring safety-sustainability trade-offs. Ultimately, the DSS aims to guide innovators from early development to market launch, fostering safer and more sustainable chemical design across Europe.

Disclaimer/Disclosure:

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6.02.P Bringing “Design” back to SSbD: Designing Molecular Structures for Safety and Sustainability

6.02.P-We405 Rational Nano-Engineering of Thyme Polyphenols Encapsulated in β -Cyclodextrin–Chitosan Carriers Mitigates Cisplatin Neurotoxicity: A Molecular-to-Organism Toxicological Assessment
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Cisplatin-induced neurotoxicity remains a major challenge in oncology, illustrating how therapeutic efficacy can be undermined by toxic consequences across molecular, cellular, and organismal levels. Aligned with the conference theme of environmental and human toxicology—from molecular mechanisms to in vivo outcomes—and the subtheme of designing safer molecular structures, this study employs a nano-engineering strategy to develop a sustainable neuroprotective formulation capable of mitigating cisplatin's damaging effects. Thyme polyphenols, known for potent antioxidant and anti-inflammatory actions yet limited by instability and low bioavailability, were incorporated into β -cyclodextrin–chitosan (CDCS) nanocarriers to enhance molecular stability, biological performance, and safety. The engineered TP#CDCS system represents a shift beyond simple substitution, focusing on structural redesign to generate a safer therapeutic adjunct.

Using an in vivo rat model with molecular toxicology assessments, we examined the ability of TP#CDCS to counteract cisplatin-induced oxidative stress, inflammatory signaling, and apoptosis. Biochemical endpoints including oxidative markers (MDA, GSH, TAC), neuronal injury indicators (CK-BB, NSE), and regulators of apoptosis and inflammation (p53, caspase-3, PGE2) were quantified to map the multi-level toxicodynamic response. Histopathological and immunohistochemical evaluations, particularly GFAP expression and neuronal architecture, provided organism-level confirmation. Cisplatin caused marked molecular and cellular disruption, whereas TP#CDCS significantly restored redox balance, suppressed apoptotic pathways, reduced astrocytic activation, and improved neuronal viability to near-control levels.

Overall, the results show that nano-engineered β -cyclodextrin–chitosan carriers enhance the functional properties of thyme polyphenols, creating a safer molecular profile and offering an effective, sustainable strategy to reduce the neurotoxic burden of a widely used chemotherapeutic agent. This work highlights the potential of combining natural polyphenols with rationally designed nanostructures to advance safer-by-design approaches that achieve therapeutic benefit while minimizing human and environmental harm.

6.02.P-We406 Redesigning Organophosphate Flame Retardants for Safety and Sustainability
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Polyurethane foams (PUF) are a ubiquitous material used in furniture, vehicles, and insulation. The flammability of PUF necessitates the use of flame retardants. After the phase-out of tris(2-chloroethyl) phosphate (TCEP) in 2015 due to reproductive toxicity and carcinogenicity, the homologue tris(chloropropyl) phosphate (TCPP) became the most used flame retardant for PUF. While sometimes assumed less toxic, a screening report from 2018 grouped TCPP with TCEP, indicating risks to children. Both chemicals are frequently found in

environmental monitoring studies.

To avoid repetition of this apparent regrettable substitution, we set out to explore molecular structure alterations to systematically improve the hazard profile and aim to design a safe and sustainable flame retardant using versatile organophosphorus chemistry. This contribution highlights the redesign process and gives an overview of the subsequent assessment.

For the molecular redesign, we employed learnings from our previous study on computer-aided redesign of organophosphates, rules of thumb, chemists' intuition and predictive models to test our hypothetical alternative structures. The design aims to decrease toxicity and increase biodegradability to prevent environmental pollution. We also explore sustainable production, linking to ongoing research in our labs to synthesize organophosphates from wastewater-recovered phosphates. The selected structure moreover contains functional groups that could allow permanent linking to matrices.

We compare our newly designed flame retardant to TCEP and TCPP, and the structurally intermediate alkyl phosphates triethyl phosphate and tripropyl phosphate, used as polymer additives. After developing a synthetic route to access our redesigned chemical, we performed a series of experiments, including (i) an investigation of the chemical stability with ³¹P-NMR, (ii) an explorative biodegradability study with sewage sludge monitoring CO₂ evolution, (iii) acute and chronic ecotoxicity studies with the algae *Raphidocelis subcapitata* and the crustacean *Daphnia magna*, (iv) ongoing evaluation of the flame retardancy of the new chemical in PUF. Results indicate that the toxicity was successfully decreased, and the degradability much increased, potentially necessitating a further design iteration to improve the balance between environmental degradability and stability during the use phase.

6.02.P-We407 Integrating Early-Stage Aquatic Toxicity Testing to Guide the Development of Recyclable, Flame-Retardant Epoxy Vitrimers

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Epoxy thermosets are widely used in electronics, construction, and transportation due to their mechanical strength, durability, and thermal stability. However, their permanent crosslinked structure limit recyclability and end-of-life options. Moreover, their flame retardancy typically relies on additive, non-reactive compounds, often halogenated, that may be persistent, bioaccumulative, and toxic. Advancing towards safer and more sustainable materials requires polymer systems that couple high performance with enhanced circularity and reduced hazard potential. Early integration of toxicological considerations is therefore a key component of the Safe and Sustainable by Design (SSbD) framework.

This study applies an SSbD-guided workflow to support the development of recyclable epoxy-based vitrimers incorporating polyphosphite and related phosphorus-containing structures. These multifunctional building blocks enable covalent adaptable networks that deliver intrinsic flame retardancy and allow thermomechanical reprocessability. However, because such structures may also contribute to environmental risks, early-stage ecotoxicological screening is critical for identifying potential concerns to enable material optimization before advancing toward upscaling.

We implemented a fish cell-based acute toxicity assay (OECD Test Guideline 249) in a 96-well plate format to increase throughput and confirmed transferability between formats using 3,4-dichloroaniline. The assay was used to evaluate the toxicity of individual monomers and cross-linkers, including diethyl phosphite (DEP) tethered to diethylene glycol (DEG), triethylene glycol (TEG), 1,5-pentanediol (PDO), and polyethylene glycol 200 (PEG-200), as well as leachates from materials under different curing and reprocessing conditions. This approach enabled systematic study of formulation, curing, and reprocessing effects, providing insights into structure–activity relationships for designing safer epoxy vitrimers.

By combining material performance data with rapid aquatic toxicity screening, the presented workflow supports iterative refinement of vitrimer formulations toward reduced hazard while maintaining essential functionalities such as flame retardancy and recyclability. Overall, this case study highlights the value of early ecotoxicological input in guiding materials innovation and demonstrates a practical approach for implementing SSbD principles in the development of next-generation flame-retardant vitrimers.

6.02.P-We408 Age-dependent ecotoxicity of biocomposites leachates towards freshwater species

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The development of biocomposites across sectors in response to environmental concerns from synthetic polymers has increased. Although promising, conventional risk assessments often overlook adverse effects.

Biocomposites may release toxic leachates with age, yet current 24-hour tests may underestimate long-term impacts. Accordingly, this work assessed the ecotoxicity of 32- and 64-day leachates from nine biocomposites [PA5%W; PA5%C; BUP04; BUP05; HTPLA-PLA-50216; HTPLA-PLA-PLA1-PLA2 (1 mm); HTPLA-PLA-PLA1-PLA2 (5 mm); HTPLA-PLA-PLA3-PLA4 (3 mm); HTPLA-PLA-PLA1-PLA4 (5 mm)] in five freshwater species: the microalgae *Raphidocelis subcapitata* (OECD 201), the macrophyte *Lemna minor* (OECD 221), the rotifer *Brachionus calyciflorus*, the cladoceran *Daphnia magna* (OECD 202), and the fish *Danio rerio* (OECD 236). Leachates were produced in distilled water following CEN/TS15863 at 8 cm³/cm² and supplemented with salts according to species requirements.

The 32-day leachates caused marked biological effects, especially at intermediate/lower dilutions for *R. subcapitata*, with sharp declines in yield and growth (e.g., 12.5–25% of HTPLA-based, PA5%C, and PA5%W composites), while minor effects were observed in *L. minor*. *Brachionus calyciflorus* showed high mortality for certain HTPLA-based and PA5%C composites, whereas *D. magna* showed no mortality. No *D. rerio* embryo mortality was observed, but larvae exposed to 100% leachates from PA5%C and HTPLA-50216 were significantly smaller than controls.

Phytotoxicity increased with ageing (64-day leachates) in *R. subcapitata*, with most composites suppressing growth at ≥25% and all causing near-zero growth at 100%, while *L. minor* effects remained minor (e.g., BUP05). *Brachionus calyciflorus* responses ranged from no toxicity to strong, dose-dependent mortality, whereas *D. magna* remained unaffected. *Danio rerio* embryos showed no mortality, but significant larval length reductions were observed for several HTPLA-based, PA5%C, and BUP04 composites, especially at 100% leachate. These findings highlight the need for ecotoxicological assessments to anticipate environmental impacts and guide biocomposite manufacturing.

6.02.P-We409 CCPD: A Promising Safer Alternative to 6PPD – Comparative Aquatic Toxicity, Environmental Fate, and Environmental Risk Assessment

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6PPD and its ozonation transformation product 6PPD-quinone (6PPD-Q) are significant aquatic toxicants, with 6PPD-Q causing acute mortality in coho salmon (*Oncorhynchus kisutch*) via stormwater runoff. In response to these findings, we evaluated N,N'-dicyclohexyl-p-phenylene-1,4-diamine (CCPD, CAS 4175-38-6) as a potential safer alternative.

This study presents environmental fate and aquatic toxicity data for CCPD and its ozonation product CCPD-Q (CAS 1653-85-6), and incorporates risk characterisation principles.

CCPD exhibits a log K_{ow} of 4.8 and QSAR-BCF value of 771. The substance demonstrated significant environmental degradation (61% biodegradation in 28 days, OECD 301D), and hydrolytic instability (DT₅₀ of 18-19 h (pH7) and 2.3 h (pH9) at 12 °C, OECD 111).

Acute toxicity to coho salmon was assessed under flow-through conditions (OECD 203). CCPD is less toxic to coho salmon than 6PPD (96h LC₅₀ of 32 µg/L for CCPD versus 14 µg/L for 6PPD). In contrast to 6PPD-Q on coho salmon, which has a 24h LC₅₀ of 95 ng/L, CCPD-Q shows no acute toxicity at the solubility limit (149 µg/L).

Compared to coho salmon, CCPD exhibited comparable toxicity to *Daphnia magna* with a 48h EC₅₀ of 58.5 µg/L (OECD 202). Acute toxicity to the common guideline fish test species, rainbow trout (*Oncorhynchus mykiss*), under flow-through conditions indicates lower sensitivity with a 96h LC₅₀ >57 µg/L (OECD 203). Additionally, recognising the relevance of pulsed stormwater exposures, a static test on rainbow trout was conducted to simulate transient exposure scenarios. The instability of CCPD and co-exposure to transformation products was apparent with a 48h LC₅₀ of 44 µg/L (EPA OCSPP 850.1075). Due to hydrolytic instability precluding standard algal tests, a flow-through *Lemna* sp. growth inhibition test (OECD 221) is also in progress. Whilst the CCPD parent compound remains toxic to aquatic organisms, it presents high potential for rapid biodegradation and exhibits elevated primary degradation rates and as such is not considered persistent.

A preliminary environmental risk assessment conducted in accordance with EU REACH indicates safe use of CCPD during manufacture and tire use, when applying the existing operational conditions and risk management measures applied for 6PPD.

These studies complete the short-term dataset for classification and hazard assessment. Future long-term studies will further define CCPD's suitability as a safer, functional alternative to 6PPD in tire and rubber goods.

6.02.P-We410 Safe-by-Design Metal-Phenolic Networks for Environmental and Biomedical Applications: A Systematic Review

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Metal–phenolic networks (MPNs), composed of plant-derived polyphenols that coordinate metal ions, are an emerging class of materials developed for applications in environmental and biomedical fields. Here, a meta-analysis of ecotoxicity data for MPNs and their constituent metal ions and phenolic ligands was conducted to support safe-by-design approaches for these materials. A systematic literature search identified 767 publications on MPNs, of which 85 contained toxicity tests without additives toxic to test organism. Most studies assessed in vitro endpoints in human or murine cells (n=75); in vivo acute or subacute tests used rodents or zebrafish (n=19). We found no environmental toxicity studies of MPNs on non-vertebrate species. Twenty-one metal ions, ten bimetallic complexes, and 29 polyphenols, plant extracts, polymers, or conjugates were reported as MPN components. MPN development for various applications has primarily focused on Fe³⁺–tannic acid systems. Fe³⁺–polyphenol complexes were generally biocompatible, whereas Cu²⁺–polyphenol complexes, which were the second most studied MPN, showed cytotoxicity. Hemolysis was examined for 23 MPNs of which three were hemolytic. No acute toxicity was observed in in vivo assays. We used harmonized hazard classifications and acceptable daily intake values of metals and ligands to assess the potential toxicity of MPNs. For environmental hazard evaluation, we collected EC₅₀ values for metal ions and phenolic ligands from 340 studies, involving algae, cyanobacteria, protozoa, crustaceans, earthworms, midges, *Aliivibrio fischeri*, and several fish species. Cu²⁺ and Cd²⁺ were consistently the most toxic ions; the toxicity of Ni²⁺, Zn²⁺, and V³⁺ varied; Ce³⁺, Gd³⁺, Al³⁺, and Eu²⁺ generally showed lower toxicity. Data on phenolic ligands were scarce, though epigallocatechin gallate has been self-classified as potentially chronically toxic to aquatic environments, while tannic acid has been classified as a GRAS (generally recognized as safe) substance. The meta-analysis aids in making informed decisions when selecting starting materials for novel MPN synthesis, upscaling, and production. The results support risk evaluation of MPNs and enable preliminary hazard ranking based on their metallic and phenolic components when empirical data on MPNs are unavailable.

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6.02.P-We411 Developing an Approach to Examine Hazards of Ingredients in Consumer Products: Application to Cleaning Products With and Without Green Labelling

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Consumers use a variety of products daily and are exposed to various chemicals contained in consumer products, for which the risks are poorly understood. While global regulatory efforts to manage chemical risks are increasing, the empirical data on persistence, bioaccumulation, and toxicity are not available for most chemicals in commerce. In this study, we selected three categories of cleaning products, i.e., all-purpose surface cleaners, laundry detergents, and dish care products. Within each category, we selected three representative products marketed as “original,” “greener,” and certified as “safer choice” by the U.S. Environmental Protection Agency (US EPA), as well as “EcoStore” from New Zealand. We focused on organic compounds in products and examined whether “greener” marketed, “safer choice”, and/or “EcoStore” products show less persistence, bioaccumulation, and/or toxicity. Data were collected using EPI Suite (US EPA) for predicted ‘P’, ‘B’, and ‘T’ values, e.g., persistence, bioaccumulation factors (BCF), and acute fish toxicity (LC50). Probabilistic distributions were developed for each P, B, and T and compared to established cut-off criteria (BCF of 500, 2000, 5000 L/kg, and LC50 of 0.1, 1, 10, 100 mg/L) to calculate exceedance probabilities. Notably, 38.8 % of the organic ingredients in “greener” labeled laundry care products exceeded the level of concern (< 0.1-1.0 mg/L), demonstrating “highly toxic” and “very highly toxic”, followed by 34.9 %, 31.2 %, and 30.4 % of “original,” “EcoStore,” and “safer choice” products, respectively. Moreover, 40.8% of the “greener” labeled laundry detergents showed the longest persistence (1/2t ? 40 days) compared to other products, which warrants potential hazards. However, more than 90 % of these ingredients were considered “low bioaccumulative (< 500 L/kg)”. Although organic ingredients in dish care products showed less toxic potential compared to all-purpose or laundry care products, 49.2 % of ingredients from the “original” labeled dish care products showed “very highly bioaccumulative (? 5000 L/kg)”. We subsequently propose a probability-based framework for comparing chemical hazards within and across specific consumer product. This approach supports data-driven substitution, encourages safer product innovation, and can be extended to broader classes of consumer products.

6.02.P-We412 Mapping the SSbD-Thinking and (lack of) Knowledge Exchange of Actors along a Product’s Life Cycle

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Safe and Sustainable by Design (SSbD) is a pre-market approach for developing chemicals that fulfill their intended functions while minimizing hazardous properties and reducing quantities that could threaten human or environmental health. The safety dimension focuses primarily on the design of the chemical or material itself, whereas the sustainability dimension considers its entire life cycle. Ultimately, these aspects and potential systemic trade-offs converge only at product-level analysis, covering the chemical's journey from its ingredient production to end-of-life treatment of the product, and potential subsequent cycles.

Various actors engage along a chemical- and product's life cycle, each with distinct interests and views on SSbD. For example, a chemist may emphasize hazard assessment, whereas a product developer focuses more on the composition of the product, material inputs, and views safety from the user perspective, and relies the chemists' assessment. This reveals a gap in understanding what SSbD entails beyond its formal definition, and how it can be operationalized. To address this, we take the product life cycle as our scope, and examine what SSbD means for different actors involved, as chemists, material developers, process designers, or product developers.

Using actor network theory, we take a socio-material approach to a case study of a care mattress. Through life cycle and actor mapping with an SSbD focus, to understand how SSbD is perceived from different perspectives. This helps illustrate how SSbD may need to be framed differently at different moments, and how it could be operationalized across a product's life cycle. We visualize scenarios with- and without SSbD thinking to clarify what SSbD entails, and what this means for the definition of SSbD. Hereby, we gain insights into actor networks and SSbD's positioning herein: where do the responsibility lie on who is doing what and when, and how does knowledge transfer through the system.

By taking this lens, we identify the different meanings of SSbD to different actors, the moments where knowledge transfer breaks down, and how to prevent the latter. We propose how SSbD can be directed to become operational (e.g., a chemist could be responsible for a thorough hazard assessment, where the product developer is responsible for consumer safety). This provides clarity to practitioners to what SSbD means for them, what information they must pass on, and where their responsibilities lie.

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6.02.P-We413 From Regulation to Data: Building an SSbD Requirements Tool Using Ontologies and Knowledge Graphs for the Pharmaceutical Sector

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Pharmaceutical innovation has greatly expanded human life expectancy, yet the life cycle of medicinal products continues to generate significant environmental and occupational challenges. Active pharmaceutical ingredients (APIs), designed to remain biologically potent at low concentrations, contribute to ecological effects and antimicrobial resistance once released into the environment. In parallel, small-molecule manufacturing is highly resource-intensive, as confirmed by LCA studies showing elevated energy demand, solvent use and waste generation, while exposing workers to potent intermediates and high-hazard solvents.

The European Commission's Safe and Sustainable by Design (SSbD) framework aims to embed safety, sustainability and environmental performance early in chemical and pharmaceutical design. However, when operationalising SSbD in the pharmaceutical sector, two major obstacles emerge: (i) SSbD requires extremely heterogeneous data, spanning toxicology, environmental fate, process safety, exposure, LCA and regulatory compliance, produced by different teams, in different formats, and at different stages; (ii) these data are governed by multiple regulatory frameworks (EU, US, OECD, ISO) that express similar requirements using inconsistent terminology, thresholds and metadata conventions. FAIR data principles are therefore essential, yet industry surveys show that data silos, non-interoperable formats and inconsistent ontologies remain pervasive barriers.

To address this, we conducted a structured policy analysis of key regulations driving SSbD-relevant evidence needs and translated deontic clauses (“shall”, “should”, “may”) into machine-readable Data Requirements. These were encoded into a cross-regulatory pharmaceutical Knowledge Graph linking regulations, requirements, datasets, lifecycle stages, ontologies and LCA-relevant parameters. Preliminary results show strong redundancy in required datasets, substantial terminology misalignment, and limited recognition of datasets shared across frameworks (e.g., biodegradation half-lives, solvent hazards, worker exposure bands). Building on this mapping, we propose the development of a data-completeness tool that prioritises high-value datasets, identifies gaps and visualises how individual data points propagate across SSbD, safety and sustainability assessments, supporting more coherent and efficient implementation of SSbD in pharmaceuticals.

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6.02.P-We414 Avoiding Tomorrow’s Chemical Mistakes Today

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The Safe and Sustainable by Design (SSbD) framework is gaining momentum within the European Union, with scholars, industry, and policymakers actively exploring its implementation. This presentation highlights key challenges that remain, particularly regarding methods, the interpretation of “life cycle” within SSbD, and data. The current methodological pillars of SSbD - Hazard Assessment (HA), Risk Assessment (RA), and Life Cycle Assessment (LCA) - offer a strong foundation for evaluating chemical safety and sustainability and for identifying potential trade-offs. However, their integration with design processes, innovation pathways, and broader ethical and social considerations remains limited. In addition, the concept of “life cycle” is not yet clearly defined in the SSbD context.

Beyond these methodological issues, the most significant barrier to SSbD is the availability and quality of data. Although notable progress has been made in advancing safety and sustainability assessment methods, improvements in data generation, accessibility, and reliability have not kept pace. Because both safety and sustainability assessments depend fundamentally on data, this imbalance weakens the effectiveness of the SSbD framework.

To address these challenges, we propose a three-step data strategy, starting with safety data. Advancing SSbD in a meaningful way requires a robust, regulatory-driven approach to enhance data availability and quality for both safety assessments and LCA. Without such a strategy, further methodological innovation risks becoming an empty exercise, one that obscures rather than enables real progress.

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6.02.P-We415 Towards Safe and Sustainable Chemical Substitution

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Chemical substitution is a critical pillar in the European Union’s Chemicals Strategy for Sustainability, aiming to mitigate human and environmental harm by replacing hazardous substances with safer alternatives. Chemicals that are persistent or induce carcinogenic, mutagenic, reprotoxic or endocrine disrupting effects are being restricted. Category-wide restrictions – such as for several PFAS - are being introduced to avoid regrettable substitution. Identifying suitable safer alternatives for restricted chemicals is challenging since an alternative should provide equal performance while being less hazardous. Information on chemical performance is specific for each application, and this information is scattered across several sources. Hazard information on chemicals is often incomplete or lacking.

We present a data-driven methodology that links desired chemical performance to physicochemical (PC) properties rather than relying solely on structural similarity, thereby reducing the risk of regrettable substitution. Target performance profiles are translated into PC property ranges, derived from benchmark substances and literature, and used to filter large candidate pools. Data gaps in PC properties and hazard are addressed using

TNO's Substance Information System (SIS), which aggregates information on >4 million chemical substances, combined with predictive in silico (QSAR/AI) models for key PC and toxicological endpoints where experimental data are lacking. This enables systematic identification and ranking of candidate substances on both performance fit and hazard indicators. Interactive visualizations (e.g. performance–hazard maps, radar plots and ranked shortlists with filters) support transparent discussions of trade-offs and selection of the most balanced options.

Case studies, including surfactant and solvent replacement, demonstrate how TNO's substitution tooling facilitates the design of products that meet both regulatory and functional requirements. This integrated approach empowers industry to advance chemical substitution, reduce hazard profiles, and deliver safe, sustainable products for global supply chains.

6.03.P Cap-and-Trade Systems for Hazardous Chemicals: Defining Caps, Allocation Weights, and Implementation Pathways

6.03.P-Mo381 A Proposal for an EU Cap-and-Trade System for Hazardous Chemicals: Design Options for SVHCs and PFAS

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The current risk management approaches under the EU's REACH regulation – restrictions and authorisation – have not been as effective in reducing chemicals-related risks as was hoped for in 2007 when the regulation entered into effect. Although the current approaches can be improved, they are likely to continue facing many of the same challenges observed today.

This paper proposes a new, complementary approach to reducing the prevalence of hazardous substances in the EU. It examines how a cap-and-trade system for hazardous substances could facilitate a significant reduction or phase-out of such substances. A cap-and-trade system – also referred to as tradeable quotas – would allow the continued manufacture, placing on the market or use of hazardous substances in cases where an outright ban would have disproportionate socio-economic impacts. By setting an ambitious reduction target for selected hazardous substances and allowing market operators to exchange allowances from one year to another or between the operators, the system would promote substitution and find cost-effective ways to reach the target.

Drawing on practical elements from existing quota and trading systems, such as the EU's Fluorinated Greenhouse Gas Regulation, the American Innovation and Manufacturing Act (of HFCs) as well as the EU Emissions Trading (ETS) of CO₂, the paper outlines how a cap-and-trade approach could be designed for hazardous chemicals. It then discusses how this system would function alongside targeted restrictions where specific risks require separate action.

The key features of any cap-and-trade system – allocation mechanisms (grandfathering or auctioning), transferability, and monitoring and verification of the quota – are discussed as well as the potential to build on the EU's existing "F-gas portal". The types of hazardous chemicals that could be regulated through a transferable quota system are also briefly examined.

A cap-and-trade system in hazardous chemicals would enable the EU to set ambitious reduction targets for the substances that are of most concern as the system would also give industry the flexibility to meet those targets in the least-cost manner. The relevance of this approach is also considered in the context of EU decision making, where adoption would follow the qualified majority procedure.

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6.03.P-Mo382 Regulating Hydrofluorocarbons (HFCs) in the EU – A Review of the Quota System

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Fluorinated greenhouse gases (F-gases) make up ~2% of total EU greenhouse gas emissions and have been regulated in the EU since 2006. Hydrofluorocarbons (HFCs) contribute ~ 90% to F-gases emissions. Next to specific bans, the 2014 revision of the F-gas Regulation (FGR) introduced an EU-wide phase-down until 2030 for HFCs placed on the market. The 2024 revision of the FGR features a steeper reduction of HFC amounts until 2030 as well as a phase-out of HFCs by 2050, accompanied by additional bans. To implement the HFC reduction, a system of HFC quotas was introduced.

HFC quota is needed by producers and importers that place HFCs on the EU market. It is allocated for a specific year based on reference values reflecting activities in previous years and on declarations of anticipated demand. Quota is valid for a specific transaction year. Quota authorisations are required when importing equipment pre-filled with HFCs into the EU. Such authorisations can be obtained from quota holders and can be banked for use in future years.

Additional flexibility mechanisms of the FGR include the possibility to transfer allocated quota and to delegate quota authorisations to other companies. However, the rules for quota transfers, quota authorisations and delegations of quota authorisation under the quota system are not designed to support free trade. Traded quota amounts are tracked in the European Commission's HFC registry, but not publicly available. Prices linked to such quota-related transactions are agreed bilaterally between involved companies and are not subject to obligatory reporting in the HFC registry. However, price monitoring data based on market research reveals significant market values of quotas and quota authorisation.

Until 2025, quota allocation was free of charge, allowing for windfall profits of quota recipients. However, the 2024 revision of the FGR introduced a fixed price of 3€ / t CO₂e, to be paid to the European Commission for the allocation of HFC quota starting 2026.

Yearly reports on reporting data by companies have shown that compliance to the system is good, with only a limited number of companies exceeding their available quota or quota authorisation. While it is not possible to quantitatively assess illegal imports of HFCs into the EU, we conclude that the FGR featuring both bans and the HFC quota system has been successful in supporting a shift in the industry away from high-GWP HFCs as well as in reducing HFC emissions in the EU.

6.03.P-Mo383 A Market for Change: Can Cap and Trade plastics systems support a more sustainable plastics future?

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In response to escalating global plastics pollution, a wide range of policy tools have been introduced across the plastics life cycle. However, as negotiations toward a global treaty progress, more integrative and flexible approaches are needed. One less explored market-based instrument that could contribute to this policy mix is a cap and trade (CAT) system. While CAT has been used in a variety of environmental contexts, its application to plastics remains unexplored. Here, we assess whether CAT can be tailored to the specific characteristics of plastics. We structure our analysis around three key design elements derived from past cap-and-trade experience: (1) defining plastic materials and target groups, (2) establishing the cap and allocating permits, and (3) creating a competitive market environment. Building on this, we explore a three-tier structure (i.e., global, national, and sectoral caps) that reflects the spatial and temporal scale of plastics across their full life cycle. Our results present viable reasons to support the potential of a plastics CAT system. It could reduce primary plastics production while also supporting recycling and fostering more circular practices by integrating economic incentives across the entire life cycle. Yet, substantial limitations persist, including the diversity of plastic materials and additives, complex transboundary supply chains, and uneven national monitoring and reporting capacities. Social and political realities, from waste-sector working conditions to the difficulty of negotiating ambitious global caps, further challenge feasibility.

Overall, our intention is not to present a CAT system as the ultimate solution for plastics, but rather to highlight that it may serve as a valuable component of a broader policy mix. By outlining opportunities and limitations, we aim to support ongoing discussions on how such a system could be designed and where it may complement future international plastics governance.

6.03.P-Mo384 Weighting Approaches for Chemicals to Develop Economic Incentives that Efficiently Reduce Hazardous Emissions

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Economic incentives are proposed as an efficient tool to motivate reduction of emissions of hazardous chemicals. If the indicators for implementing such a market-based system are science-based, with weighting approaches to compare chemicals, the data can also support decision-makers opting for less hazardous alternatives. Weighting of chemical properties and/or hazard classifications, that are often associated with large knowledge gaps, is not without complication. Therefore, it is important to critically assess previous work to learn from those examples. Here, different economic instruments to reduce the use of hazardous chemicals, and the underlying weighting approaches, have been reviewed and assessed with respect to feasibility and extendibility beyond the sectors and chemical domains to which they currently apply. The pros and cons of using different weighting-approaches have been analysed to develop scenarios for possible implementation of science-based economic instruments, targeting different environmental compartments and chemical groups. The weighting-approaches were categorised in five different groups: (1) flat rate-based, (2) hazard-based, (3) impact-based, (4) risk-based, (5) allocation-based approaches. A diverse number of methods, fitting into the weight-approach categories, were identified at different spatial and temporal resolution. The different approaches have differing requirements on chemical data (physicochemical properties, (eco)toxicological profile, and environmental fate properties) and current approaches rarely consider the real-world complexity of chemical exposures but rather assessing chemicals and their effects in isolation. The data availability and assessment will partly determine the optimum choice of economic instrument, where a flat-base rate can be assigned as a measure of per-kg chemical emitted/produced while a quota system would also require scientific assessments of the carrying capacity of the exposed environmental compartment (or human). Due to the heterogeneity and uncertainty of chemical impact, the boundaries of the system need to be clearly defined and the procedure of weighting of chemicals must be transparent. Effective economic instruments can complement the current regulatory framework and increase incentives for reducing the emissions of hazardous chemicals.

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6.03.P-Mo385 Using Relative Risk Indicators to Inform Allocation Weights in Chemical Cap-and-Trade Systems

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Cap-and-trade systems for hazardous chemicals can complement existing regulations by creating market incentives for earlier and more efficient substitution. A central requirement is the development of allocation weights that reflect differences in the potential to cause harm across substances. This work examines how Relative Risk Indicators (RRIs) from the Environmental Risks and Impacts of Chemicals (ENRICH) framework can inform such weights.

ENRICH assigns substances to eco-potency groups using species sensitivity distribution data for more than 12 000 chemicals and to exposure groups using national and international monitoring distributions. These groupings form a 7×7 matrix of RRIs that represent substance-specific levels of concern based on combined eco-potency and exposure. Because RRIs are ordinal, they indicate which substances are likely to pose greater concern relative to others, but they do not define the absolute magnitude of risks or impacts.

To explore how RRIs can be translated into numerical allocation weights, three illustrative weighting rules are developed: (i) a community-impact rule based on predicted percentages of species affected within the ENRICH framework, (ii) a multiplicative rule using the product of eco-potency and exposure groups, and (iii) an additive rule using their sum. Each rule is normalised to allow comparison of the degree of separation they create between levels of concern, i.e., how strongly it differentiates quota requirements across substances with different RRIs.

The three approaches generate distinct patterns of regulatory pressure. Community-impact weighting creates steep differences between substances with high RRIs and all others, concentrating compliance costs on those of greatest concern. Multiplicative weighting spreads cost differences across the full range of RRIs, strengthening substitution incentives for both mid- and high-concern substances. Additive weighting offers only limited separation, simplifying implementation, but provides weaker substitution signals for the higher RRIs than the

other two approaches.

This analysis illustrates how RRI grouping can be operationalised into alternative allocation weighting rules and provides a basis for further methodological development of cap-and-trade mechanisms for chemicals.

6.03.P-Mo386 The possible role of financial incentives in the substitution of very hazardous substances

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Regulatory authorities at both the Belgian national level and the European level have concluded that the current pace of chemical substitution is insufficient. Apeiron-Team supported two studies commissioned by these authorities and investigated barriers and incentives for substitution. These studies were informed by real-world substitution cases and interviews with stakeholders included manufactures and users of the incumbent, alternative suppliers, investors and NGO's next to the regulators. One key measure identified was the introduction of financial incentives. This concept was further developed to promote safer and more sustainable chemical use while safeguarding the competitiveness of European industry.

This presentation outlines the development of such financial instruments and highlights areas for further refinement. The concept, grounded in the polluter-pays principle, primarily targets industrial applications, focusing initially on downstream users and manufacturers. In practice, the greatest leverage for substitution is expected among downstream users, for whom the concept has been most extensively developed.

A central consideration is that the financial mechanism should not hinder innovation. To address this, partial fee waivers could be granted based on the implementation of efficient innovation programs. Efficiency may be demonstrated through collaborative efforts to eliminate hazardous substances while emissions and exposure are reduced beyond legal requirements.

The fee is calculated using reported emission and exposure data combined with monetized impact assessments, similar to those used in authorization dossiers. A key area for further exploration is the integration of life-cycle impacts, as current authorization assessments focus primarily on direct emissions and exposure. Tools such as ProScale, evaluated within Safe and Sustainable by Design (SSbD) projects, are being considered for this purpose.

The concept could also incorporate the availability of alternatives, significantly increasing the financial fee where substitution is feasible. The proposed "avoidable use fee" aims to reduce the impact of highly hazardous substances by imposing a proportional financial burden on continued use when safer alternatives or impact-reduction measures are achievable through innovation.

6.03.P-Mo387 Cap-and-Trade Systems for Hazardous Chemicals – What Can We Learn from F-Gas Regulations in the EU and the US?

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Cap-and-trade systems have been widely used in climate policy but remain largely unexplored for regulating hazardous chemicals. Two existing systems, the EU Fluorinated Greenhouse Gas (F-gas) Regulation and the US American Innovation and Manufacturing (AIM) Act, govern industrial chemicals through transferable quotas and therefore provide relevant experience for considering similar approaches for hazardous substances. This paper reviews how these systems function and identifies design features that may be applicable when regulating groups of hazardous chemicals.

Both systems establish legally binding, declining caps on the placing on the market or consumption of hydrofluorocarbons (HFCs). The 2024 revision of the EU F-gas Regulation introduced a much steeper reduction trajectory than the earlier 2014 regulation, reflecting rapid technological progress, strengthened EU climate targets, and the need for improved market surveillance. Verified data exist for quota allocations and placing-on-the-market quantities, while emissions trends rely on inventory estimates rather than direct compliance monitoring.

Transferability of allowances is permitted in both jurisdictions. In the United States, the Environmental Protection Agency publishes annual notices confirming that allowance transfers occur. In the European Union, transfers are recorded in the F-gas Portal, but aggregated trading volumes are not publicly available. Evidence suggests that such transfers facilitate compliance but do not constitute a large, liquid market.

Several elements of these systems appear relevant for potential cap-and-trade approaches to hazardous chemicals: robust inventories before setting the cap; multi-year allocation windows; clear long-term reduction

schedules; centralised and audited reporting; and transferability to allow substitution to start where costs are low. While hazardous chemicals present additional challenges due to diverse hazard profiles and use patterns, the experience from HFC quota systems demonstrates that quota-based approaches can provide predictability, flexibility, and cost-effective pathways for reducing harmful substances.

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6.04 Endocrine Disruption: Scientific and Regulatory Challenges

6.04.T-01 Characterisation of molting disruptors in arthropods using an AOP-informed multi-species screening approach

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Molting is a central developmental process in arthropods, governed by coordinated endocrine, neuroendocrine, metabolic, behavioral, and structural pathways. These pathways are susceptible to disruption by chemicals that interfere with hormonal signaling, cuticle turnover, chitin synthesis, neuropeptide activity, or gene regulatory networks. Because molting disruption can arise through diverse and often poorly resolved mechanisms, new mechanistically anchored tools are needed to identify key events underlying toxicity and to detect molting disruptors across a broad range of arthropod species and chemicals.

This project, co-financed by the Partnership for the Assessment of Risk from Chemicals (PARC), aims to establish an integrated, AOP-informed scientific framework to characterise molting disruption using model organisms and wildlife species. The work involves sequential steps starting with a systematic review, refinement, and expansion of existing molting-relevant Adverse Outcome Pathways (AOPs). Conserved molecular initiating events, key events, and pathway linkages will be mapped and evaluated across taxa, while also identifying knowledge gaps for targeted experimental investigation.

Building on this mechanistic foundation, a suite of bioassays has been developed and will be harmonised across laboratories to quantify molecular, cellular, phenotypic, behavioral, and organism-level responses to stressors. Approaches include transcriptomics, targeted gene expression analyses, metabolomics, hormone quantification, behavioral profiling, and advanced in vitro and in vivo NAMs. Computational x-species comparisons will support identification of conserved responses and improve extrapolation across species groups.

The resulting toolbox will be applied to environmentally relevant chemicals to identify compounds capable of perturbing molting physiology and related biological pathways. Multi-endpoint and multi-species datasets will be integrated to construct quantitative AOPs (qAOPs), enabling the development of predictive models and quantitative key event relationships describing how early molecular perturbations propagate to organism-level adversity for use in Next Generation Risk Assessment (NGRA).

This presentation will summarise the scientific foundation, introduce the methodological approaches, and present results from initial steps of the work (i.e. AOP-definition, bioassay development, reference chemical testing).

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6.04.T-02 RetiNAM: Identification of Biomarkers for the Disruption of the Retinoic Acid Signaling Pathway Using a Zebrafish Embryo Model

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Endocrine-disrupting chemicals are a major concern in ecotoxicology due to their ability to interfere with hormone signaling across species. Therefore, current regulations for chemicals such as REACH, have a particular focus on the identification of endocrine disruptors (ED). Standardized test methods for endocrine disruption focus on EATS (estrogen, androgen, thyroid, steroidogenesis) modalities. The retinoic acid (RA) pathway is another highly conserved endocrine signaling pathway, essential for physiological and cellular processes including neurodevelopment and morphogenesis. As RA pathway disruption can affect organisms at population-relevant levels, regulatory interest in the retinoid system has grown, with OECD efforts in 2012 and 2021 to review and harmonize regulations, focusing on key organ systems and highlighting the need for validated assays. However, there is a lack of validated methods to detect disruption of the RA signaling pathway. In line with the EU "Roadmap to phase out animal testing", new approach methods (NAMs) are needed to fill ED-related testing gaps. One strategy to identify RA disrupting chemicals is the identification of biomarkers for key events leading to apical adverse outcomes. The RetiNAM project aims to identify and quantify sensitive and specific biomarkers for RA pathway disruption in zebrafish embryos with a focus on chemicals that act via binding to the retinoid X receptor (RXR) or retinoic acid receptors (RAR), and elicit transcriptional changes leading to morphological and behavioral outcomes. We demonstrated that exposure to RAR and RXR agonists and antagonists induced specific effects, including craniofacial, fin and tail malformations. Acute neuroactivity testing indicated diverse neuroactive effects across multiple endpoints. Developmental neurotoxicity testing resulted in altered visual and acoustic startle responses. Additionally, widespread hyper- or hypoactivity were observed, respectively. These results suggest that the zebrafish embryo model can be used to diagnose RA pathway perturbations. Ongoing transcriptomic analyses will identify key RA pathway target genes, anchoring the phenotypic effects to the underlying molecular mechanisms. By integrating morphological assessment, behavioral profiling and transcriptomic analysis, this study will address current regulatory gaps in the evaluation of RAR or RXR-mediated endocrine disruption and supports the use of NAMs in environmental risk assessment.

6.04.T-03 Which Fish? Considering Inter-Species Differences in the Design of a Fish Full-Life Cycle Study with an Estrogen Receptor Degradar

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Imlunestrant is a potent, selective estrogen receptor alpha (ER α) degrader approved for breast cancer. To evaluate environmental risk from patient use, a fish full-life cycle study (FFLC) was essential. Draft and validated FFLC guidelines vary in recommended fish species but do not consider underlying biology. Since physiological roles of ER and pharmacological specificity vary between fish species, we investigated imlunestrant's species-specific ER antagonist activity and developmental/reproductive effects across three small fish models to determine if one species was substantially more sensitive and establish appropriate test levels. Initial ER antagonist assays revealed marked interspecies variability in response to imlunestrant. Zebrafish showed IC₅₀ values of 128.3 nM (ESR1), 14.05 nM (ESR2a), 3.85 nM (ESR2b). Medaka demonstrated IC₅₀ values of 17.52 nM (ESR1), 20.42 nM (ESR2a), 34.61 nM (ESR2b). Fathead minnow exhibited IC₅₀ values of 25.37 nM (ESR1) and 0.553 nM (ESR2b), 100-fold lower than other species; ESR2a was not identified in fathead minnow at the time of this work. The paucity of data for ER knockout in fathead minnow hindered interpretation, so in vivo studies were conducted.

In vivo species sensitivity screening (21-day adult reproductive assays plus embryo-larval assessment) at nominal concentrations of 1.5, 15, and 150 μ g/L imlunestrant correlated with receptor sensitivity. Fathead minnow showed pronounced reproductive effects (31% and 79% reduction at 15 and 150 μ g/L, respectively) and concentration-dependent reductions in larval growth (13 to 32% weight reduction). Zebrafish and medaka showed minimal reproductive impacts in adults and no differences in larvae.

Based on sensitivity results, a FFLC (OECD 240) was conducted with fathead minnow. Fish were exposed continuously across three generations to five measured concentrations ranging from 0.015 to 8.1 μ g/L (F0), 0.019 to 7.1 μ g/L (F1), and 0.022 to 6.9 μ g/L (F2). At sexual maturity, the F1 generation showed significant

effects at 7.1 µg/L including reduced hatching (14%), increased male vitellogenin (80-fold), and increased length/weight in females. Males from F1 reproductive groups showed increased weight at 1.8 µg/L, the most sensitive endpoint. Gonadosomatic index and interstitial cell proliferation increased significantly at 7.1 µg/L, confirming endocrine modulation. Overall NOEC and LOEC were 0.43 and 1.8 µg/L.

6.04.T-04 Assessment of thyroid disruption using an alternative fish model, the three-spined stickleback

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Regulatory assessment of chemicals with endocrine-disrupting properties covering oestrogen, androgen and steroidogenic pathways, are conducted traditionally using three model fish species, zebrafish, medaka and fathead minnow. However, investigations for chemicals that disrupt the Thyroid Hormone System (THS) employ amphibians.

A detailed review paper (DRP) prepared for the OECD proposed a series of biochemical, histological, and morphological endpoints for assessing THS-disrupting chemicals in fish, originally developed for zebrafish under an EU funded project, ERGO. The authors joined the OECD validation work for these endpoints and PARC, where they were tasked on strengthening the AOPs for thyroid disruption in fish, focusing on TR antagonism, a less developed molecular initiating event (MIE).

The studies presented evaluated the responses of the three-spined stickleback (*Gasterosteus aculeatus*) to two prototypic stressors, in view of expanding the taxonomic domain of applicability of fish thyroid AOPs. Exposures involved the natural thyroid receptor (TR) agonist, thyroxine (T4) and the TR antagonist, 1-850 whilst endpoints were assessed at TG236 (Fish Embryo Toxicity Test) and TG210 (Fish early life toxicity test) stages (12dpf and 40dpf respectively for this model species).

In addition to the proposed endpoints under OECD validation, we assessed behaviour and spatial gene expression, both at eleuthero-embryo level. The results are presented along with apical and thyroid specific endpoints from juvenile fish (TG210), discussing the value of these endpoints for regulatory testing and AOP development.

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6.04.P Endocrine Disruption: Scientific and Regulatory Challenges

6.04.P-Mo388 A Desk-Based Approach to Determine the New CLP Endocrine Disruption Hazard Classes

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With the implementation of new endocrine disruption (ED) hazard classes in the CLP regulation (Regulation (EC) No 1272/2008) and increasing regulatory scrutiny under REACH (EU & UK), the Biocidal Products Regulation (BPR) and the Plant Protection Products Regulation (PPPR), ED assessment of substances/actives must be proactively addressed to ensure compliance and market access.

Based on expert judgement through developed knowledge and experience as well as key guidance documents, we developed a flow scheme for the stepwise approach of assessing ED properties based on available and alternative information. This tiered approach, from Phase I initial screening, to Phase II full ED assessment, aids in the fulfilment of the requirements under the new CLP hazard classes, avoids additional animal testing, and aligns with state of the art of regulatory science.

In short, Phase I includes a literature search to identify possibly relevant data from open literature, an evaluation of the literature to retrieve relevant and reliable information, database searches and in silico profiling. These lines of evidence are collected in a structured excel file, which allows for the generation of Conceptual Framework (CF) Level 1 information in accordance with the ECHA/EFSA Guidance (2018). The next step is then to screen for adverse effects to human health and environmental species that may be plausibly linked to an endocrine mode of action and thus contribute evidence for the substance to be classified as an endocrine disruptor. Phase I is concluded with a screening report summarising the available data, its interpretation and any conclusions. The process either stops if no effects of relevance are identified or moves to a Phase II full ED assessment if required. Specifically, our long-standing expertise in in silico profiling underpins this regulatory compliance focused screening and assessment procedure by aiding robust conclusions on ED modalities and negating the need for additional animal testing. Here we show the flow scheme and a case study of how the lines

of evidence go toward a robust and coherent conclusion on ED.

The presented approach ensures compliance with the new CLP requirements for hazard communication on endocrine disruption based on all available data, proactively identifying potential requirements asked for by authorities, extending the longevity of submissions.

6.04.P-Mo389 Beyond EATS: How to Handle Non-EATS Data in an Endocrine Disruption Weight of Evidence Assessment

Chloe Eastabrook and Gareth Le Page, Enviresearch, United Kingdom

The recent update to ECHA's Classification, Labelling and Packaging (CLP) guidance introduced two hazard categories for endocrine disruptors (ED). As CLP does not require additional testing for hazard classification there is an emphasis on the importance of weight-of-evidence (WoE) approaches. Thus, assessments rely on existing data, often from the open literature where reliability and relevance can vary significantly.

Current regulatory focus has largely centred on EATS-mediated pathways (estrogen, androgen, thyroid, and steroidogenesis). However, the updated CLP guidance also acknowledges non-EATS mechanisms despite providing no guidance on how to define these or how they should be evaluated. Non-EATS data will likely originate from studies without standardised test guidelines and might include non-standard species, adding further complexity to interpretation. While non-EATS modalities were mentioned in the ECHA EFSA 2018 guidance for pesticides and biocides, the CLP update has brought renewed attention to these often less-characterised modalities.

Integrating non-EATS data into regulatory assessments presents challenges, including the lack of standardised testing frameworks, limited adverse outcome pathway (AOP) development beyond EATS, and the need for robust criteria to assess relevance and reliability. This poster will discuss these additional challenges to an already highly complex assessment process. Alongside this, practical approaches for scoping and incorporating non-EATS data and modalities into ED assessments will be explored, ensuring current EU regulatory requirements are met.

6.04.P-Mo390 Endocrine Disruption Assessments – Where Yesterday's Submissions Meet Today's Expectations

Eva Eschenbach and Florian Zindler, Exponent, Germany

The evaluation of substances for potential endocrine disrupting (ED) properties in non-target organisms is a key component of chemical risk assessment. However, only in 2018 these assessments became mandatory and around the same time, EFSA and ECHA published a joint guidance document designed to support the selection of appropriate testing strategies and the evaluation of conducted studies. Since then, both applicants and regulators have gained substantial experience in the evaluation of ED studies and accompanying assessments, and the overall knowledge base has expanded significantly.

Given that many submissions were originally prepared at a time when regulatory expectations and experience were still limited, while the evaluation of active substance renewals can extend over several years, a common challenge arises: Studies and accompanying assessments were often submitted when experience was still developing, yet they are reviewed later against a much broader and more mature body of case examples. As a result, discrepancies frequently occur between the applicant's conduct and interpretation of the data at the time of submission and the regulators' evaluation at the time of review. Furthermore, the scientific literature continues to expand rapidly, and applicants often receive requests from stakeholders to assess the relevance of newly published peer reviewed studies for ongoing renewal processes.

Due to the procedural constraints of the renewal process, opportunities to address comments from the regulators and other stakeholders after submission are limited, particularly when additional information, new studies or extensive literature evaluations are required to resolve outstanding concerns.

It is therefore crucial to review existing ED assessments and studies for completeness and relevance against the current state of the (regulatory) science. To this end, we propose strategic approaches for proactively managing potential uncertainties within submitted ED studies and assessments and provide recommendations for the systematic evaluation of relevance and reliability of scientific literature. The aim is to better anticipate regulatory requests, strengthen the scientific robustness of submissions, and minimise the need for additional testing. Insightful examples are provided to highlight the untapped potential of study raw data, the importance of historical controls, and the practical application of recent scientific and regulatory recommendations.

6.04.P-Mo391 Assessment of Endocrine Effects of Construction Products in the Environment

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Endocrine disruptors are substances that interfere with the hormonal systems of humans and animals and can cause harmful effects even at extremely low concentrations. They influence vital processes such as reproduction, development, growth, and behavior. These endocrine-disrupting chemicals (EDCs) are present in many synthetic products – including construction materials, whose hormonal effects are often insufficiently understood because no comprehensive testing is required before they are placed on the market.

Considering this background, there's an urgent need to systematically investigate EDC-related risks and to identify alternative construction products that are safe for human health. This serves consumer protection and supports the EU's goals within the framework of the circular economy and the zero-pollution strategy. The contamination of groundwater and especially of drinking water as a potential consequence with EDCs released by construction products should be prevented in future.

The aim of the research project is to evaluate construction products regarding potential endocrine effects in environmentally exposed applications. The basis for this is the European testing standard DIN CEN/TS 17459:2023-02, which provides guidance for the assessment of the release of hazardous substances via eluates. However, this standard currently does not include any tests for detecting endocrine effects.

The project therefore examines the suitability of commercially available, particularly standardized endocrine test methods (such as ISO 19040-3 for estrogenic activity, ER CALUX) for the investigation of construction-product eluates. The objective is to develop scientifically sound recommendations for expanding the testing methodology to include appropriate endocrine assays.

In addition, proposals will be developed on how endocrine effects can be integrated into the certification criteria of Blue Angel ecolabel to make safe products identifiable to consumers.

Work Package 1 involves a literature review on suitable endocrine tests for aqueous samples and on hormonally active substances in construction products intended for outdoor use. This review forms the basis for a testing program in Work Package 2, which includes 20 construction products and four laboratory tests for detecting endocrine effects. Product selection is based on environmental relevance, while the tests consider both acute and chronic effects as well as scientific, practical, and economic aspects.

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6.04.P-Mo392 From Literature Search to Regulatory Assessment: Addressing Endocrine Disruption with Non-Standardized Data

Imke Van Dingenen¹, Finn Thomson¹, Nele Deleebeeck², Michiel Claessens¹ and Bart Kerré¹, (1)Arcadis, Belgium, (2)ArcelorMittal, Belgium

In 2023, new hazard classes for endocrine disruption (ED) were introduced under the European Classification, Labelling and Packaging (CLP) Regulation (Regulation (EC) No 1272/2008). All substances and mixtures products placed on the EU market must be classified according to these new hazard classes by the applicable deadline. As CLP does not require the generation of new test data, companies and regulators should rely exclusively on existing data.

In the current project, a comprehensive literature search was conducted to determine the current knowledge on potential ED activity and adverse effects for a series of rare earth elements (REEs). One of the main challenges for these types of literature searches is the lack of harmonized guidance for assessing the reliability and regulatory adequacy of data derived from non-standardized studies. Therefore, available guidance documents (e.g. OECD guidance document 150 on the testing and assessment of endocrine disruption, ECHA/EFSA guidance, CLP and REACH endpoints specific guidance) were used to extract relevant criteria to develop a tailored strategy and template for systematically assessing the reliability of literature data on endocrine disruption properties. Key aspects addressed include the evaluation of study design (e.g., test species, endpoints measured), limitations in the study design, relevance to ED mechanisms (such as estrogenic, androgenic, thyroid, and steroidogenic pathways). The proposed template and strategy represent a step forward in bridging this regulatory gap and may serve as a foundation for future harmonized approaches in the evaluation of non-standardized ED studies.

This project highlights the need for further regulatory and scientific guidance on the assessment of non-standardized ED data to facilitate the effective use of available evidence for regulatory decision-making under

the revised CLP Regulation. Improved guidance will be essential to ensure both the protection of human health and the environment and the efficient management of substances lacking standardized data, such as REEs.

6.04.P-Mo393 Weighing the Real Evidence and Avoiding the ED Trap

Nicola Dennis and Stephen Ruckman, Sagentia Regulatory, United Kingdom

Since 2018, it has been a requirement for active substance renewal and new active substance registration to provide an assessment on the endocrine disrupting properties of a pesticide in accordance with EFSA / ECHA Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107 / 2009. In a bid to harmonise assessments for endocrine disruption, an expert meeting was held in November 2024, a report on the outcome of this meeting was published in October 2025 (Recurring issues in mammalian toxicology and ecotoxicology related to endocrine disruptors. EFSA Supporting publication 2025:EN9743). Common challenges in the interpretation of non-target organism data were identified and include:

- High variability and poor reporting of vitellogenin (VTG) a biomarker used to identify EAS (estrogen, androgen and steroidogenesis) activity in fish.
- Quality of reporting for histopathological evaluations.
- Data limitations leading to a limited weight of evidence and uncertainty.
- Limited experience in performing and interpreting Conceptual Framework Level 5 data.

We present supportive evidence to open up the discussion on the challenges of interpreting endocrine disruptor data, particularly when faced with identifying a plausible link between adversity and endocrine activity when an alternative non-endocrine mode of action may be the driver. Focussing primarily on the EAS modality, we will discuss how variability in VTG data can lead to uncertainty. Particularly when looking at the influence and relevance of possible outliers. Should effects only be considered at the replicate level even if this seems improbable at the individual level. Can histopathologists reliably opine on the potential for endocrine disruption without sight of the whole dataset. How is uncertainty balanced when looking at the weight of evidence particularly when considering a non-endocrine mode of action. Is there sufficient knowledge within Industry and Regulatory Authorities to critically assess alternative pathways that may be based on years of targeted academic research. How easy is it to move away from the tiered approach of testing under the Conceptual Framework to generate data to answer specific questions.

6.04.P-Mo394 Is it time to revisit threshold levels for endocrine disruptors?

Rebecca Jayne Brown and Grace Panter, wca environment Ltd., United Kingdom

The European Union (EU) approach for assessment of most chemicals with an endocrine disrupting (ED) mode of action is to assume there is no safe level of exposure. ED hazard assessment has been adopted based on legitimate concerns regarding the potential for low concentration responses (LCR) and non-monotonic concentration responses (NMCR) following exposure to some endocrine active substances. Although hazard-based regulation has improved understanding of ED modes of action there are concerns that it relies on intensive animal testing, focuses on effects at high concentrations, can be time consuming to reach a conclusion and can lead to unfortunate substitutions. In addition, it limits potential for exposure-based waivers for low volume or readily biodegradable substances which will never be present in the environment at meaningful concentrations and is a barrier to adoption of exposure based next generation risk assessment approaches. Standardisation of fish endocrine toxicity tests (increased replication, advice on concentration setting, minimising solvent use, etc.) has improved confidence in their outcomes and the routine inclusion of biomarker endpoints means that there is supporting information regarding mechanistic effects and concentration response relationships. Case studies with estrogenic substances, azole fungicides and pharmaceuticals are used to demonstrate that NMCR and LCR are not the rule and that it is possible to derive threshold levels for many ED substances. This provides a basis for rethinking regulation of ED substances to allow a more pragmatic approach in scenarios where exposure is negligible.

6.04.P-Mo395 Assessment gaps and opportunities in regulatory endocrine disruptor testing of fish – a scientific perspective

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Regulatory assessment of endocrine disruptors (EDs) in fish relies largely on established OECD test guidelines (TGs), yet these protocols make limited use of the biological information that could be obtained from exposed

animals. While EU chemical regulation increasingly requires animal testing for ED identification, the upcoming EU roadmap for phasing out animal experiments aims to achieve the opposite. This is problematic, as for the identification of EDs an adverse effect, an endocrine activity, and a biologically plausible link between the two has to be demonstrated. However, demonstrating endocrine adversity currently still requires data from animal experiments and cannot (yet) be generated through new approach methodologies (NAMs) alone. Improving existing fish tests is therefore essential as the currently established OECD in vivo TGs often fail to exploit the full potential of animal experiments.

We summarized endpoints included in validated OECD TGs using fish and compared them with additional endpoints that could technically be assessed but are not currently required. Across 91 possible endpoints, only 31% are mandatory in present TGs, leaving 69% of available biological information unused. The gaps span molecular, physiological, morphological, and behavioural levels. Despite their routine use in research and diagnostic laboratories, transcriptomic analyses, hormone measurements, expanded histopathology of organs beyond the gonads, and systematic behavioural assessments are still not included in fish regulatory tests. Their integration would substantially strengthen mechanistic interpretation, support identification of adversity, and increase the scientific robustness of ED evaluations without increasing animal use.

This simple but insightful analysis demonstrates that there is a strong potential to improve regulatory decision-making and ensure more ethical, scientifically sound use of animals in ED assessment by including multiple relevant endpoints assessed with existing routine methodologies.

6.04.P-Mo396 Best Practices on the Conduct of The Fish Sexual Development Test (FSDT) – OECD TG 234 – Part 1

Francesca Pellizzato, Conor Clenaghan, Helene Serra-Niemininen, Pamela Campbell, Marta Sobanska and Vincent Bonnomet, European Chemicals Agency (ECHA), Finland

The Fish Sexual Development Test (FSDT) according to the OECD TG 234 is a validated test guideline targeting early life stages of development to assess effects on vitellogenin (VTG) and sex ratio. It provides information on both the endocrine activity and adversity of potential endocrine disruptors (ED). It is the main test requested under REACH in the substance evaluation process to clarify endocrine disrupting properties related to estrogenic, androgenic and steroidogenic modalities in fish. The test can also be requested under REACH during the compliance check process instead of the fish early life stage test (OECD TG 210) if there are triggers that the substance is potentially an ED. The information obtained from an OECD TG 234 study may provide conclusive evidence on the ED properties of a substance leading to its identification as a Substance of Very High Concern under REACH and/or its classification as an ED under the classification, labelling and packaging (CLP) Regulation. Since its adoption in 2011, the test has been requested by Member States or ECHA in more than 20 decisions under REACH. Because it requires testing on vertebrate animals, it is important to ensure that the test is performed as requested and provides reliable information. Using the accumulated experience on FSDT studies, an ad-hoc working group was established in 2025 by Member States and ECHA to review the test performance and address the main challenges identified. The analysis focused on 15 studies received under REACH and conducted by 8 different laboratories: 13 performed with zebrafish and 2 performed with Japanese medaka. Based on that analysis and following the discussions in the ad-hoc working group, a technical note has been prepared outlining key requirements and recommendations for the conduct and reporting of FSDT studies according to OECD TG 234.

This poster presents Part 1/2 of the technical note, which provides clarifications on specific requirements of the test guideline - including duration of the test, redistribution of the fish, sex ratio and exposure concentrations for difficult-to-test substances - to improve the performance of the studies. A separate abstract has been submitted (Part 2), which covers practical recommendations to support the performance and reporting of the test.

6.04.P-Mo397 Best Practices on the Reporting of the Fish Sexual Development Test (FSDT) – OECD TG 234 - part 2

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The Fish Sexual Development Test (FSDT) according to the OECD TG 234 is a validated test guideline targeting early life stages of development to assess effects on vitellogenin (VTG) and sex ratio. It provides information on both the endocrine activity and adversity of potential endocrine disruptors (ED). It is the main test requested under REACH in the substance evaluation process to clarify endocrine disrupting properties related to estrogenic, androgenic and steroidogenic modalities in fish. The test can also be requested under REACH during the compliance check process instead of the fish early life stage test (OECD TG 210) if there are

triggers that the substance is potentially an ED. The information obtained from an OECD TG 234 study may provide conclusive evidence on the ED properties of a substance leading to its identification as a Substance of Very High Concern under REACH and/or its classification as an ED under the classification, labelling and packaging (CLP) Regulation. Since its adoption in 2011, the test has been requested by Member States or ECHA in more than 20 decisions under REACH. Because the test requires testing on vertebrate animals, it is important to ensure that it is performed as requested and provides reliable information. Therefore, with this accumulated experience, an ad-hoc working group was established by Member States and ECHA to review the test performance and address common challenges. The analysis focused on 15 studies received under REACH, 13 using zebrafish and 2 using Japanese medaka, performed by 8 different laboratories. Based on that analysis and the discussions in the ad hoc working group, a technical note has been developed outlining key requirements and recommendations for the conduct and reporting of FSDT studies according to OECD TG 234.

This poster presents Part 2/2 of the technical note, focusing on practical recommendations for study performance and reporting. This part of the note supplements the requirements in the OECD TG 234 by including further guidance on the selection of test concentrations, evaluation and documentation of histopathological endpoints, and assessment of vitellogenin levels. A separate abstract has been submitted (Part 1), which addresses specific requirements of the test guideline.

6.04.P-Mo398 Does age matter?: An Investigation of the Variation in Vitellogenin Production Compared to Fathead Minnow (*Pimephales promelas*) Age Using Historical Control Data

Amy Snow, Smithers, United States

Vitellogenin (VTG) is the egg yolk precursor protein produced by the liver in fish, a process thought to be mediated by estrogen, androgen and steroidogenic (EAS) modalities. Test guidelines for potential endocrine disruptors have integrated this biomarker (in combination with other weight of evidence) as a method of detecting interference with the endocrine system. The highly variable data produced from this indicator has been shown to be a challenge in endocrine screening assays as decreased statistical sensitivity is prevalent. The variability of VTG concentration among fish of the same sex has been attributed to several different factors (e.g. naturally occurring variability, spawning cycle timing, etc.). This investigation utilized historical control data to compare the plasma VTG concentrations from both male and female fathead minnow (*Pimephales promelas*) in order to evaluate the variability in comparison to the age of the fish. Data from Fish Short-Term Reproductive Assays, Fish Sexual Development Tests and Fish Full Life Cycle Tests (modified to include endocrine endpoints) were analyzed in order to determine a potential correlation between VTG concentration and age. In addition, potential correlations between other variables (e.g. wet body weight and fecundity) were explored. This analysis aims to understand a potential cause for the variability in VTG concentrations of sexually dimorphic Fathead Minnow to potentially inspire changes in guidance for future testing.

6.04.P-Mo399 Body weight change in Fish Short Term Reproduction Assays

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The Fish Short Term Reproduction Assay (FSTRA) is a screening assay for the Estrogen, Androgen and Steroidogenesis modalities in fish. Sexually mature spawning male and female fish are held together and exposed to a chemical for 21 days. Symptoms of toxicity, fecundity and fertility are assessed daily. At termination vitellogenin, secondary sexual characters (species dependent) and gonad histopathology are analysed. Measurements of wet weight and length are taken before the exposure to ensure the fish are within a suitable size and variability range. The OSCPP test guideline also requires measuring body and gonad weights at termination to calculate the Gonadal Somatic Index. It has been demonstrated that many of the mechanistic endpoints may be affected by generalised toxicity or are correlated to body size. Therefore, it is of high interest to understand how body weight change can be assessed in range finding and FSTRA studies to improve test designs and interpretation of results. In analogy to how such assessments are used in mammalian toxicology studies. Here, we analyse the results of a historical dataset of FSTRA studies to explore what range of weight changes have been observed and if there is any relationship with effects on the FSTRA endpoints. We then use a recently performed FSTRA dietary restriction study to explore the power of assessing body weight change. Recommendations for implementing body weight change in FSTRA studies are made. We hope these analyses will support further use of body weight data in the performance and interpretation of FSTRAs conducted for regulatory and research purposes.

6.04.P-Mo400 Why not Medaka?: An Overview for the use of Japanese Medaka (*Oryzias latipes*) as a Model Species for Endocrine Disruption Investigation Using Historical Control Data

Tracey Jayne Goodband, Smithers, United Kingdom

Over the last decade, more emphasis has been placed on the investigation of potential endocrine activity of chemicals on fish with new requirements under EFSA/ECHA and US FDA/EPA. Standard OECD in vivo guidelines for investigating potential endocrine activity in fish include the Fish Short Term Reproduction test (OECD 229), Fish Sexual Development Test (OECD 234) and the Medaka Extended One Generation Reproduction Test (OECD 240). The Japanese Medaka (*Oryzias latipes*) is recommended as a test species in these guidelines and is specific to the OECD 240 (MEOGRT), which is the only Tier 5 test for aquatic organisms.

The small size, relatively short life cycle, easily identifiable phenotypic secondary sex characteristics and genetic sex determination makes *O. latipes* an excellent species for laboratory testing. However, it is not a species that has been traditionally used in Europe or America with most CROs preferring fathead minnow and/or zebrafish. As such, there is a lack of experience with the species and historical control data (HCD) is limited. This presentation will discuss HCD data collected at Smithers ERS over the last 7 years from studies conducted with medaka with specific emphasis on the MEOGRT.

6.04.P-Mo401 Medaka and Zebrafish Display Contrasting Responses to Chronic Exposure to Ethinyl Estradiol

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Medaka and zebrafish have become standard models to study the impacts of endocrine disruptors on fish. Although these two species of fish share many similarities and advantages, there are important differences in terms of sex differentiation which could influence the results of studies relating to the reproductive axes. To determine the sensitivity of physiological responses of the two species to estrogenic chemicals, we conducted two parallel chronic exposures of both medaka and zebrafish with ethinyl estradiol (EE2). The fish were exposed from fertilisation to 60 days post hatch in an OECD test guideline 234-like exposure. Fish were then reared in clean water for 3-4 months. Endpoints were measured at both the end of the exposure phase and the end of the depuration phase and included physical condition, mortality, gene expression, gonadal sex, reproductive competence and F1 larval survival.

In response to EE2, medaka exhibited an increase in the expression of genes regulated by estrogenic signalling such as vtg and ER α , increased incidence of intersex in genetically male fish, a female biased sex ratio and reduced F1 survival. Expression of vtg and ER α was also increased in zebrafish exposed to EE2. However, after the exposure period, there appeared to be a delay in sex differentiation in zebrafish in response to EE2 and following the depuration period, adult zebrafish exposed to 10 or 30 ng/L EE2 were all phenotypically male. As it was impossible to determine genetic sex with this strain of zebrafish, it is unclear whether the male skewed sex ratio was due to EE2 provoking stress-induced masculinisation of fish that would have developed as female and/or selective mortality of female fish. Histopathological analyses of juvenile zebrafish (30 ng/L) revealed very high amounts of proteinaceous fluid (presumably VTG) in kidney tubuli. Excessive hepatic VTG production can reach levels that exceed renal filtration capacities, leading to death. These effects may have caused female-specific mortality.

Metabolomic studies may also give insights into whether increased mortality and delayed sexual differentiation are linked to increased energy expenditure due to over stimulation of the production of glycoproteins required for oocyte production following exposure to exogenous estrogens. It remains to determine to what degree these effects on sex differentiation are due directly to endocrine signalling and whether they are representative of wild-type ZZ/ZW zebrafish.

Disclaimer/Disclosure:

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6.04.P-Mo403 Assessment of Thyroid Hormone System Disrupting Effects of the Insecticides Diflubenzuron and Pyriproxyfen During Early Zebrafish Development

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Mosquito larval control in the Global South relies on insect growth regulators such as diflubenzuron (DFB) and pyriproxyfen (PPF), which the World Health Organization recommends as a vector control strategy. Although these larvicides exhibit low acute toxicity, they may still affect non-target aquatic organisms at sublethal concentrations, possibly acting as endocrine disruptors. This study employs a recently developed Adverse Outcome Pathway (AOP) framework-based experimental approach to assess whether DFB and PPF act as potential thyroid hormone-disrupting chemicals (THDCs) in fish. Using Tg(tg:mCherry) zebrafish (*Danio rerio*) embryos, we measured swimming behavior and thyroid follicle development after 96 h of exposure to DFB and PPF (nominal concentrations of 1, 2, and 4 mg/L). Both compounds led to a significant increase in thyroid follicle fluorescence intensity, indicating changes in thyroid development and activity. Light–dark behavioral testing revealed increased swimming during the light phase and decreased swimming during the dark phase, suggesting neurodevelopmental effects and/or altered visual processing. These results are consistent with the AOP proposed by Götz and colleagues (2022), in which thyroid peroxidase (TPO) inhibition leads to retinal layer changes that can affect visual function and swimming performance in fish. Ongoing histopathological analyses of retinal structure aim to determine whether DFB and PPF exposure had direct impact on eye development, the key event of the AOP which would support their classification as THDCs. Additional assessments will examine potential neurotoxic effects of DFB and PPF in the Tg(eno2:Cerulean, gfap:mCherry, mbp:citrine) triple-transgenic line and estrogenic effects in the Tg(vtg1:mCherry) line. These findings will provide detailed insight into the effects of DFB and PPF on non-target organisms and thereby provide critical evidence to guide the development of future environmental regulations on larvicide use in the Global South.

Disclaimer/Disclosure:

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6.04.P-Mo404 Identification of Histopathological Reference Points in the Brain of Zebrafish Embryos to detect Morphological Changes induced by Endocrine Disruptors

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Thyroid hormone system-disrupting chemicals (THSDCs) can interfere at different level of the Hypothalamic-pituitary-Thyroid (HPT) axis, and exposure during early life stages can lead to different adverse effects, such as impaired neurodevelopment. As the HPT axis is highly conserved among vertebrates, zebrafish embryos provide a powerful model for studying thyroid disrupting effects. The Thyroid-Fish Embryo toxicity (tFET), was developed in the ERGO project and is currently under OECD validation. This assay covers multiple endpoints related to THSD (TH levels, swim bladder inflation, eye development, and thyroid follicle morphology using a transgenic line).

Data from tFET experiments performed in the ERGO project show that exposure to different model THSDCs during early life stages (from 0 to 120 hpf) impaired normal eye development. To assess this adverse effect, histopathology was used to measure the thickness of retinal layers and to assess morphological changes in eye development (see AOP#365). During those analyses it was observed that the THSDC exposure also had an effect on brain development of exposed zebrafish embryos, an effect that is well established in mammals. Consequently, as a follow-up to the work conducted in ERGO, the present study aims to investigate potential morphological alterations in the developing brain using the same histological sections.

The first step to establish such an assessment is to identify reliable histopathological reference points in different regions of the brain of zebrafish embryos to subsequently detect and measure morphological changes that are comparable between individuals.

To investigate brain development across multiple regions, specific histological sections were selected based on the presence of distinct morphological structures that could serve as reliable reference points. These reference points were chosen from different region of the brain to allow detection and comparison of morphological changes across regions.

By identifying consistent reference points within the embryonic brain, additional quantitative information can be extracted, including the grey-to-white matter ratio in different brain regions and total brain area relative to overall embryo area. This approach could provide valuable evidence of neurodevelopmental impairment caused by THSDC exposure and may additionally help to determine whether it exerts region-specific effects on brain development.

6.04.P-Mo405 Endocrine Disruption of Zebrafish Brain and Sensory Organ Development

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Thyroid hormones (THs) are crucial for neurodevelopmental processes of vertebrates, making them potential targets of endocrine disruptors. Accordingly, impaired neurodevelopment is the primary adverse health effect of THSDs in humans, while it has not been sufficiently addressed from that perspective in fish. A recently established adverse outcome pathway (AOP) shows the link between lowered TH levels and impaired eye development resulting in impaired vision and lowered survival of fish (AOP #365). However, the impact of TH system disruptors (THSDs) on other neurodevelopmental processes, such as brain and sensory organ formation have not sufficiently been studied in fish. Consequently, the present project aims to fill knowledge gaps on TH-regulated development of the fish brain and sensory organs, such as the olfactory epithelium and the lateral line. The findings will be set into an environmental context by investigating the impact of THSDs on development and function of these important parts of the nervous system in developing fish embryos. This will be done according to the AOP concept to identify critical neuro-related endpoints for THSD in fish that are also relevant for risk assessment of other vertebrates and humans. Different transgenic zebrafish lines with fluorescently labelled nervous and sensory cells will be exposed to different model THSDCs to study the impact on specific neurodevelopmental processes from molecular up to behavioural level.

Disclaimer/Disclosure:

The PhD project will start in December 2025. First results will be presented at the 36th SETAC Europe annual meeting in May 2026.

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6.04.P-Mo406 Brain Morphology Impairment in Zebrafish Embryos as an Endpoint of Thyroid-related Developmental Neurotoxicity

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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 the thyroid hormone system (THS) during early-life development. 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 a triple-transgenic zebrafish (*Danio rerio*) 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. In this study we focus on the oligodendrocyte differentiation as a potential DNT endpoint. Previous studies have shown alterations in oligodendrocytes differentiation following exposure to several DNT compounds and THSDCs. Consequently, we are investigating exposure to different THS disruption mechanisms of action, that likely result in structural alterations of oligodendrocytes.

After 5-days of exposure to model compounds, T3 and Propylthiouracil (TH synthesis inhibitor), we firstly investigated oligodendrocytes structural alterations in zebrafish embryos by confocal imaging. Results show that the volume of oligodendrocytes in the brain decreased in PTU-exposed fish and increased in T3-exposed fish. Secondly, to confirm dysregulation in key genes expression involved in neurodevelopmental processes, we are investigating thyroid- and brain-related gene expression and DNT biomarkers. After validation of the methods using the model compounds, two environmental relevant pollutants, known for their THS disrupting properties, triclosan and benzophenone-2, will be tested in this model. These new brain-specific approaches will be combined with specific TH-related endpoints as TH levels and thyroid follicle morphology to assess sensitivity and specificity of oligodendrocytes imaging.

In summary, our data suggest that new endpoints focusing on DNT could be a great addition for THS disruption assessment in zebrafish embryos, as the recently developed tFET (thyroid Fish Embryo Toxicity test) currently under OECD validation.

6.04.P-Mo407 Screening ANSES priority substances for thyroid activity using two Eleuthero-embryonic Bioassays

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Chemical disruption of thyroid function can occur through multiple modes of action (MoA), and there is a growing need for whole-organism assays capable of detecting diverse mechanisms while remaining aligned with the principles of animal welfare. Two complementary eleuthero-embryonic tests have been developed for this purpose: the *Xenopus* Eleuthero-embryonic Thyroid Assay (XETA), OECD test guideline n°248, based on a transgenic *Xenopus laevis* line expressing GFP under a thyroid-responsive promoter, and the Transgenic Eleuthero-embryo Thyroid Specific test (TETHYS), based on a transgenic medaka line expressing GFP in thyroid follicles under the thyroglobulin promoter. Both assays operate over 72 hours and make use of non-protected developmental stages, offering an ethical and rapid screening strategy.

As part of the French national effort to strengthen the evaluation of endocrine disruptors, ANSES established a priority list of 16 chemicals requiring targeted testing because of suspected endocrine-related effects, environmental occurrence, or human exposure potential. This list includes industrial chemicals, pesticides, metabolites, and transformation products for which in vivo endocrine data are limited or insufficient for regulatory decision-making. In this study, these priority substances were screened for thyroid activity using both the XETA and TETHYS assays.

XETA primarily detects MoA affecting thyroid hormone signaling, while TETHYS provides complementary sensitivity to mechanisms involved in thyroid hormone synthesis, including iodine-uptake inhibition. Among the substances tested, four demonstrated clear thyroid activity: two were positive in XETA and two in TETHYS. These findings highlight the mechanistic complementarity of the two assays and the value of applying them in parallel to improve the detection of thyroid-active substances. For each active chemical, preliminary MoA hypotheses were generated by comparing the profiles obtained with those of previously characterized reference compounds.

Overall, this study demonstrates that combining XETA and TETHYS provides a robust and ethically improved approach for identifying thyroid-active chemicals. This dual-assay strategy enhances MoA coverage and supports more reliable prioritization.

Disclaimer/Disclosure:

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6.04.P-Mo408 Bridging In Vivo and In Vitro Evidence from New Approach Methodologies (NAMs): Comparing XETA and ToxCast Data to Identify Thyroid-Active Substances

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The identification of thyroid-active chemicals remains a major challenge for hazard assessment. The thyroid system can be affected through various mechanisms, including hormone synthesis, metabolism, and receptor-mediated pathways. To improve mechanistic understanding, it is essential to establish the coherence between in vivo and in vitro New Approach Methodologies (NAMs) targeting the thyroid axis.

The *Xenopus* Eleutheroembryonic Thyroid Assay (XETA) is an OECD test guideline (OECD TG 248) for identifying thyroid active chemicals. The XETA uses fluorescent *Xenopus laevis* transgenic eleutheroembryos to identify activity of the thyroid axis and how it can be altered by chemicals. As it uses non-protected embryonic life-stages, according to the current EU legislation on the protection of experimental laboratory animals, the XETA is considered a NAM.

In the present work, we conducted XETAs with reference chemicals selected with existing knowledge on their specific mechanism of action in mammals to expand the dataset of reference chemicals that were used in the OECD validation of the test guideline. In total, 60 reference chemicals have now been tested in the XETA. These substances were selected to represent a broad range of mechanisms, including thyroid peroxidase inhibition, thyroid hormone synthesis disruption, altered deiodinase activity, and competitive binding to thyroid hormone transport proteins.

To further explore the mechanistic concordance between in vivo and in vitro NAMs, we have interrogated the CompTox Chemicals Dashboard containing relevant high- throughput bioactivity data (ToxCast and Tox21 databases) to retrieve assay data for the same set of chemicals. We have then performed a comparative analysis to evaluate the level of agreement between the responses in the XETA and the high- throughput in vitro

mechanistic signatures.

This combined analysis aims to (i) assess the reliability of the XETA in capturing thyroid-related mechanisms of action; (ii) identify consistencies and discrepancies with in vitro assay outcomes; and (iii) contribute to the integration of mechanistic data in a weight-of-evidence approach for endocrine disruptor identification. Ultimately, this work will help clarify how amphibian-based embryo assays and large-scale in vitro screening platforms can be used to support the implementation of NAMs and provide confidence in the assessment of thyroid-active substances within regulatory contexts.

Disclaimer/Disclosure:

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6.04.P-Mo409 Impact of a Mixture of Organic Pollutants on the Thyroid Axis

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Endocrine disruptors are widely recognized for their adverse effects on human and environmental health. Studies suggest that their ubiquitous presence impact amphibian metabolism, contributing to their decline over several decades. In this context, a mixture of six obesogenic EDs (TBT [organotin compound], TCS [chlorophenol / antimicrobial agent], DDE [organochlorine pesticide metabolite of DDT], PBDE [brominated flame retardant], BaP [polycyclic aromatic hydrocarbon – PAH], and DEHP [phthalate plasticizer]) was assessed at concentrations corresponding to environmental quality standards (EQS) during short- and long-term exposure in *Xenopus tropicalis* at three developmental stages (NF47, 62, and 65). We hypothesized that metabolic dysregulation observed on liver by our collaborators could find some origins in brain and thyroid gland, given that the thyroid system plays a key role in metabolic regulation.

Regarding the F0 generation, short-term exposure (4 days post fertilization; NF47) induced an increased cell proliferation and reduced cell mortality in the brains of exposed tadpoles. We assessed mobility of animals with light/ dark stimulus. *X. tropicalis* eleutheroembryos were naturally more active in darkness, and exposure significantly intensified this mobility. At the molecular level, using RNAseq, exposure to the mixture led to deregulation of several genes involved in metabolism and thyroid function. In the long term (3 weeks), histological analyses on premetamorphic (NF62) and metamorphic (NF65) tadpoles revealed pronounced alterations in the thyroid glands, including increased colloid content, clusters of thyrocytes, and follicular disorganization, which were observed significantly in both sexes.

To investigate potential transgenerational impacts of this obesogenic mixture, the F1 generation which was not directly exposed was also examined. Long-term exposure (3 weeks) resulted in a significant decrease in thyroid colloid in both male and female tadpoles (NF65), and the observed pattern suggests a possible maternal transmission of this impairment.

Overall, these results demonstrate that this obesogenic mixture is biologically active even at EQS concentrations, highlighting the need to assess chemical mixtures rather than isolated compounds in environmental risk evaluation. Notably, thyroid impairments were observed in non-exposed offspring, revealing a transgenerational impact that may have important implications for long-term population health.

6.04.P-Mo410 Optimising the First Phase of OECD Guideline 241: the Larval Amphibian Growth and Development Assay to meet Validity Criteria and Analyse Plasma Thyroid Hormone Levels.

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The Larval Amphibian Growth and Development Assay (LAGDA) is a level 4 in vivo assay of the OECD Conceptual Framework on Endocrine Disruptors Testing and Assessment and provides data on adverse effects on the hypothalamus pituitary thyroid and gonadal axes using the model *Xenopus laevis*. The assay was adopted in 2015 with few chemicals tested during the validation process and little data on inter-laboratory reproducibility. So far, only a few studies have been conducted and very few laboratories are able to perform this test.

Starting at Nieuwkoop and Faber (NF) stage 8 to 10 for approximately 110 days depending on control animals' developmental rate, the study is divided in two phases, larval and juvenile, with a period of transition with duplicated replicates. A previous full scale trial performed in our laboratories highlighted difficulties with

maintaining adequate water quality, especially during the larval phase of the study, due to the feeding regime proposed in the guideline. Also, regulatory authorities often request additional endpoints such as thyroid hormones plasma analysis which needs developing.

The aim of this study was to perform a feeding trial focused on the first phase of LAGDA following the OECD 241 guideline up to NF stage 62 by trying different feeding regimes with different flow rates to investigate the feasibility of passing strict study validity criteria. The study was initiated with four sets of two replicate dilution water control vessels with 20 *X. laevis* eggs. An adapted diet of sera® Micron following 50% of recommended feeding guidelines in test guideline OECD 231 as well as an enhanced sera® Micron diet supplemented with *Artemia* spp. nauplii and spirulina was used. A flow-through design with 35 ml/min and 50 ml/min flow rates were also tried. Blood samples were collected at NF stage 62 and analysed for Thyroid Stimulating Hormone (TSH), free and total T3 (triiodothyronine) and T4 (thyroxine) using analytical method developed and validated in-house. Data was used to determine typical control levels, required minimum sample volumes and level of dilution. Crucial information regarding the quantity of blood drawn from NF stage 62 animals was gathered to determine if successful analysis of all hormones in a single individual was possible.

6.04.P-Mo411 When Tadpoles Grow Faster Than Expected, Statistics Get Tricky

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When tadpoles develop very fast, standard statistics can fail. We show how to rescue OECD TG 231 analyses with a practical, reproducible nested ANOVA strategy.

The Amphibian Metamorphosis Assay (OECD TG 231), occupying Level 3 in the OECD Conceptual Framework for endocrine disruption testing, tracks developmental changes over 21 days, from (Nieuwkoop–Faber) NF stage 51 to ~NF 60. Endocrine diagnostic endpoints include developmental stage (NF) and normalized hind limb length (nHLL) at days 7 and 21, while wet weight (WW) and snout to vent length (SVL) are also recorded and analyzed.

Under typical conditions, ~80% of tadpoles per group remain at NF <60 and endpoint means are analyzed using standard methods. But when >20% surpass NF 60 in one or more groups, the guideline's Annex 3 prescribes a two factor ANOVA with nested variance. This framework uses all tadpoles, groups them by LateStage (NO: NF <60; YES: NF >60), and “slices” the analysis into separate ANOVA F tests for each LateStage group. Annex 3 significantly increases the complexity of the required analysis. To perform this analysis correctly and thoroughly, we see a need for a more thorough explanation. We've also identified and corrected inconsistencies and inaccuracies in the methodology.

Key statistical insights include:

1. Correction of the appropriate error structure to avoid pseudo replication.
2. What is slicing?
3. Considering monotonicity assumptions in concentration–response trends.

We also address common obstacles:

- Lack of mixed model capability in available software.
- Software limitations for slicing.
- Absence of LateStage = YES tadpoles in controls.
- Missing LateStage categories in certain treatment groups.

6.04.P-Mo412 Thyroid Hormone Disruption by Model Chemicals and Indoor Dust: A Multi-Country Assessment

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Endocrine-disrupting chemicals (EDCs) pose significant risks to human health. In addition to dietary sources, environmental exposure contributes substantially, particularly indoors, where limited ventilation and multiple pollutant sources create complex chemical mixtures. The indoor environment is therefore a critical exposure setting, especially for young children, who are more vulnerable due to ongoing development and behaviors such as floor contact and hand-to-mouth activity. Disruption of the system of thyroid hormones (TH) is of particular concern since it can affect crucial processes such as metabolism regulation, neurodevelopment, reproduction, and carcinogenesis.

This study applied a battery of new approach methodologies (NAMs) using in vitro bioassays to evaluate TH-related endocrine-disrupting potential in a diverse set of model chemicals relevant to human exposure. These chemicals represented multiple categories, including pharmaceuticals, pesticides, industrial compounds, environmental pollutants, and constituents of personal care and consumer products. Additionally, complex mixtures extracted from 21 indoor settled dust samples collected from households with young children in four European countries were assessed.

The assay battery targeted key TH-related endpoints: synthesis (thyroperoxidase [TPO] inhibition), transport (thyroxine–transthyretin [T4–TTR] displacement), receptor agonism (ThR), and metabolism (deiodinase inhibition [DIO1, DIO3], and aryl hydrocarbon receptor [AhR]-mediated activity). Endocrine-disrupting potential was detected in most tested chemicals and dust sample extracts. Notably, settled dust extracts exhibited diverse effect patterns across countries, with frequent detection of T4–TTR displacement and AhR-mediated activity.

These findings underscore the pervasive endocrine-disrupting potential of indoor environments and the complexity of identifying causative pollutants, highlighting the need for improved risk assessment strategies for indoor exposure.

Disclaimer/Disclosure:

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6.04.P-Mo413 Inhibition of the Thyroid Hormone Receptor Alpha (TR α) by Mycotoxins and Their Mixtures

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The effect of mycotoxins on thyroid hormone receptor alpha (TR α) has been poorly studied. Only beauvericin (BEA), ochratoxin A (OTA), and deoxynivalenol (DON) have been evaluated showing antagonistic effects. Furthermore, mycotoxins frequently co-occur in aquafeeds, which may lead to synergistic or antagonistic interactions.

This study aimed to evaluate the effect of mycotoxins commonly found in aquafeeds on TR α . First, twelve substances were individually tested, including the acetylated derivatives 15-O-acetyl-DON and 3-acetyl-DON, aflatoxins (AFB1, AFB2), enniatins (ENNA, ENNA1, ENNB, ENNB1), fumonisins (FB1, FB2), nivalenol (NIV) and zearalenone (ZEN). In addition, the combined exposure of BEA, ENNB and FB1 was evaluated. For this study, the PC-DR-LUC cell line was exposed 24 hours to the mycotoxins, both in the absence and in presence of triiodothyronine (T3), to determine whether they induce or inhibit TR α activity. The mycotoxins were tested at a range of non-cytotoxic concentrations. For the co-exposure assays, cells were treated with two fixed concentrations of ENNB or BEA (0.1 μ g/mL and 1 μ g/mL, non-effective and effective, respectively) combined with FB1 (0.019-10 μ g/mL). The co-exposure effect of ENNB and BEA was assayed in a range of ENNB concentrations (0.0049-1.25 μ g/mL) with the two fixed concentrations of BEA.

None of the tested mycotoxins showed agonistic activity on TR α . No antagonist effects were recorded for 3-acetyl-DON, 15-acetyl-DON, AFB2, ENNA, FB1, FB2 and ZEN. However, NIV presented a strong antagonistic activity, followed by ENNB1, ENNA1, ENNB and finally AFB1. Co-exposure assays revealed that all concentrations of FB1 reverse the inhibitory effect produced by ENNB at 1 μ g/mL. FB1 and BEA generate a double response following a U-shaped hormetic response. FB1 increased the inhibition of the receptor produced by BEA (0.1 μ g/mL) at low concentrations (0.078-2.5 μ g/mL) and reverse the antagonistic effect produced by BEA (1 μ g/mL) at high concentrations (1.25-10 μ g/mL). The inhibitory effect induced by BEA at 0.1 μ g/mL was enhanced by ENNB at concentrations of 0.62 and 1.25 μ g/mL. Also, the effect of BEA at 1 μ g/mL was increased by ENNB at the highest non-toxic concentration assayed (1.25 μ g/mL).

These results highlight the need to evaluate the mixture effects due to the coexistence of multiple mycotoxins in aquafeeds. Understanding how they interact is essential to protect fish health and aquaculture production.

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6.04.P-Mo414 A Tiered Strategy for the Integration of Complementary Lines of In Silico Toxicological Evidence Permitting Evaluation of Endocrine Disrupting Potential

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A variety of computational modelling tools, including quantitative structure-activity relationships (QSAR), structural alert-based profilers and molecular docking simulations, are available for the purposes of providing evidence in relation to toxic potential. Confidence in the appropriateness of their conclusions may be enhanced by means of considering the weight-of-evidence drawn from an assortment of approaches – particularly where these should align in description of a credible adverse outcome pathway (AOP) linking molecular initiating event (MIE) to apical effect.

Within this study, a tiered testing strategy, allowing for the rational integration of outputs emerging from a collection of complementary *in silico* techniques, is described. The context explored is that of endocrine disruption (ED) – a complex collection of outcomes, holding significance within both human and environmental toxicology. Although challenging to characterise with sufficient reliability by means of individual tools, existing mechanistic knowledge may be harnessed in order to provide a basis for the compilation of evidence.

The preliminary phase of the strategy concerns evaluation of the suitability of existing experimental data, with respect to the substance and endpoint of interest (for example, oestrogenic, androgenic, thyroid-mediated effects). Should available data be insufficient, or absent, then *in silico* screening is enacted. Relevant profiling tools are initially applied, with the intention of identifying compounds bearing structural features liable to be associated with the given effect. Absence of such alerts would see that, as a secondary means of examination, appropriate QSAR were utilised. If likewise negative, then a conclusion of lower ED concern may be drawn. Hits at either the profiler or QSAR stages shall precede the application of molecular docking approaches, relatable specifically to MIE. Evidence of strong interaction with the protein target may be interpreted as indicative of putative ED potential – itself further examinable by means of more expansive quantitative AOPs (qAOPs) based upon suitable *in vitro* data.

Presented case studies demonstrate the applicability of the tier-based assessment strategy. Furthermore, they allow for identification of challenges associated with such integrated approaches – most notable amongst which are the need for validation procedures, alongside a capacity to appraise the levels of certainty required to make a decision.

6.04.P-Mo415 Quantitative Structure–Activity Relationship Models for Assessing Thyroid Hormone System Disruption: A Review

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Thyroid hormone (TH) system disruption is a significant health concern, due to the critical role THs play in metabolism, growth, and brain development. Traditional reliance on animal-based testing for hazard assessment is no longer feasible, driving the need for new approach methodologies (NAMs), such as *in silico* methods based on quantitative structure–activity relationship (QSAR) approaches. This review, covering scientific literature from 2010 to 2024, maps the state-of-the-art in QSAR model development for predicting TH system disruption. A specific focus is placed on QSAR models that address molecular initiating events (MIEs) within the adverse outcome pathway (AOP) framework for TH system disruption to place the review at the science-policy interface.

A total of 30 papers presenting 86 QSAR models are analyzed, examining their data sources, modeled endpoints, algorithms, molecular descriptors selected, validation strategies, and the definitions of their applicability domains (ADs).

Modelled datasets fall into two categories: those including structurally heterogeneous chemicals (67% of the QSARs), and class-specific datasets mainly focused on halogenated and/or hydroxylated structures that resemble THs (e.g., PCBs, PBDEs), limiting the number and variety of local QSARs. QSARs primarily target TH receptors (TRs) and transthyretin (TTR), which together account for 57% of all models reviewed. A major gap remains, as many critical MIEs among the over 20 identified in the literature are either significantly under-addressed or entirely unmodeled. Despite the consistent application of validation procedures, a crucial finding is the frequent lack of a clearly defined AD for approximately 37% of the QSAR models, which severely limits their utility. Furthermore, while key structural features driving TTR and TR binding are consistently identified, there is limited emphasis placed on mechanistic interpretation of models, which can undermine their reliability. This review serves as a "state-of-the-art" resource, identifying both existing QSARs and critical research gaps. The findings underscore the immediate need for increased data generation for poorly addressed MIEs, broadening chemical space coverage beyond TH-like structures, and prioritizing models with clearly defined

ADs and enhanced mechanistic interpretability. Advancing these areas will promote wider confidence in and regulatory acceptance of QSARs as effective NAMs for assessing TH system disruption

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6.04.P-Mo416 A Clustering Approach for Profiling Potential Estrogen Receptor-Mediated Endocrine-Disrupting Chemicals

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Endocrine-disrupting (ED) chemicals influence the normal function of the endocrine system through diverse mechanisms, including binding to, activating (agonism), or blocking (antagonism) nuclear hormone receptors (NRs). Estrogen Receptor (ER), which belongs to NRs, is among the primary known targets of endocrine disruption.

Although ED chemicals have been widely reported over decades, the identification and detection of substances with ED properties (e.g., estrogenic activity) remain a challenging task. They represent a highly heterogeneous group of chemicals, each characterized by distinct chemical properties and biological activities (bioactivity). Considering this diversity, endocrine disruptions do not have a single endpoint. It requires multiple assessment methods to evaluate the interaction between chemicals and NRs. However, testing all compounds with standard biological-based tests is not feasible.

The present study aims at proposing an *in silico* clustering approach to categorize the ED potency of compounds by linking their chemical structures and bioactivity. Targeting the estrogen pathway, we tested the hypothesis that structurally similar compounds, which would be assigned to the same cluster, might have similar estrogenic ED potency. TyPol (Typology of Pollutants), an in-house web platform, was used to perform clustering based on Partial Least Squares (PLS) regression and a hierarchical clustering algorithm. A total of 81 molecules constituted the ER agonist data set. The model was built using 42 molecular descriptors (e.g., molecular weight...) and *in vitro* ER bioactivity (i.e., relative binding affinity (RBA), 10% of maximum effect (EC10)). To assess the specificity of endocrine-mediated activity, we calculated the specificity ratio (SR_{baseline}). Then, we computed the Bemis-Murcko scaffolds for each compound to analyze chemical similarity within the cluster. Our initial findings suggest that the TyPol tool can discriminate between organic compounds having higher ED potency and those having lower potency. Clusters with higher estrogenic ED potency contain molecules that share flavones (e.g., quercetin, genistein) and steroidal scaffolds (e.g., mestranol, estriol). They are characterized by moderate to high RBA, a lower EC10, and a higher SR_{baseline} compared to other groups. The four-component PLS model indicates good statistical performance. This research could support improved decision-making on screening and prioritization of ED chemicals.

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6.04.P-Mo417 Mixture Effects of Bisphenol A and Analogues on the Regeneration of Freshwater Planarians

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Bisphenols are synthetic compounds widely used in the production of polycarbonate and epoxy resin polymers, with Bisphenol A (BPA) being the most common. Due to its endocrine-disrupting properties, BPA has been increasingly replaced in consumer products by structural analogues. However, many of these alternatives exhibit comparable or even greater toxicity than BPA and have been detected in various environmental matrices.

Because environmental exposures occur as complex mixtures, evaluating their combined effects is crucial for realistic hazard and risk assessment. The growing use of BPA alternatives, coupled with significant data gaps regarding their single and mixture toxicity, makes this an urgent regulatory concern. Freshwater planarians, recognised for their remarkable regenerative capacity, provide a sensitive model for assessing the effects of chemicals that may interfere with tissue regeneration, such as endocrine disruptors. Therefore, this study aimed to investigate the effects of bisphenol mixtures on the regeneration of the freshwater planarian *Girardia tigrina*. Adult planarians were decapitated immediately before exposure to BPA, BPAF, BPS-MAE and BPZ, and their binary mixtures for eight days. Mixtures were prepared following a fixed-ratio ray design. Regeneration of the blastema was measured at 48 hours, and photoreceptor and auricle regeneration were monitored every 24 hours. Data were analysed using the MixTox tool, applying the Concentration Addition (CA) and Independent Action (IA) models, with tests for deviations indicating synergism, antagonism, or dose-dependent effects. Among the individual compounds, BPAF was the most toxic ($EC_{50} = 0.40$ mg/L), followed by BPZ ($EC_{50} = 0.83$ mg/L), BPA ($EC_{50} = 3.54$ mg/L) and BPS-MAE ($EC_{50} > 5$ mg/L). The BPA + BPAF mixture was best described by the CA model, suggesting additive effects and a similar mode of action. In contrast, the BPS-MAE + BPZ mixture was best fitted by the IA mode, indicating distinct modes of action and limited interaction. The results enhance our understanding of the single and mixture effects of BPA analogues, which are crucial for addressing key gaps in hazard assessment and preventing regrettable substitutions. Moreover, *G. tigrina* proved to be a sensitive model for evaluating regenerative disruption by endocrine-active compounds.

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6.04.P-Mo418 Effect-Based Methods (EBM) and Effect-Based Trigger (EBT) values for Estrogenicity Monitoring in Surface Water: an Interlaboratory Study

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Effect-based methods have been included in the Water Framework Directive to evaluate estrogenic substances. The European Commission's Joint Research Centre performed an interlaboratory study to assess and evaluate estrogenic effect-based methods and effect-based-trigger values to figure out if they would be compliant with chemical analysis. To do so, the effect-based-trigger values were derived based on three options: linking the effect-based trigger value to environmental quality standards (option 1), correlating in vitro and in vivo data (option 2), and averaging bioassay-specific effect-based-trigger values (option 3).

In this study, surface water samples from eight Northern Italian sites with known estrogenic hormone and endocrine-disrupting chemical contamination were analysed by fourteen laboratories using effect-based methods, and four performed chemical analysis.

Chemical analysis suggested a cumulative risk of estrogenic hormones and endocrine-disrupting chemicals in several samples with estrone and bisphenol A as main drivers. Effect-based methods detected estrogenic activity, though specificity varied: some bioassays responded selectively to hormones, while others also detected other endocrine-disrupting chemicals. Notably, effect-based methods identified estrogenic risk in a sample

where chemical analysis showed no individual exceedances of environmental quality standards. The highest compliance with chemical analysis across water samples was achieved when effect-based trigger value option 1 was applied.

The study also highlighted the need to harmonise the effect-based methods – including data evaluation – for addressing chemical mixtures, with recommendations to the Member States for their application in the Water Framework Directive.

In conclusion, effect-based methods demonstrate high sensitivity in detecting estrogenic effects and their integration with chemical data ensures protection against cumulative estrogenic risks from both hormones and endocrine-disrupting chemicals. However, the coverage of mode of action remains limited for other pollutants, and in vitro assays capable to detect multiple modes of action – such as those critical for assessing per- and polyfluoroalkyl substances - are often lacking. Biotechnology-based innovations demonstrate significant potential to advance this field by accelerating efforts to close the existing gap.

6.04.P-Mo419 Development of a Highly Sensitive Reporter Gene Cell Line for Detecting Estrogenic Activity (the ER Isjaki Assay)

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In recent years, the European Union has proposed changes to the regulation for environmental protection of water bodies by including the environmental quality standards (EQSs) for 17 β -estradiol (E2), estrone (E1) and 17 α -ethinyl estradiol (EE2) in inland and other surface waters, which are below the chemical limit of detection. Although bioassays based on mammalian cells have generally lower detection limits than chemical analysis for estrogens, the suggested EQSs are also challenging the analytical capacity of the current bioassays. These changes highlight the need to develop more sensitive assays capable of detecting estrogens at regulatory relevant concentrations. As a result, this study aims to develop and establish the optimal experimental conditions for a novel and highly sensitive reporter-gene assay designed to detect estrogenic activity.

To this end, optimization of transfection plasmid concentration, exposure time and basement membrane matrix effect, assay reproducibility and quality as well as relative effect potency (REP) of natural estrogens and estrogenic compounds were carried out. The optimal conditions for the newly developed assay based on transiently transfected MCF-7 cells were set as 5 ng of plasmid per well in 96-well uncoated plates and exposure to treatments for 24 hours. As a result, the assay displayed an average effect level 20%, also expressed as sensitivity, for E2, E1 and EE2 of 0.29, 1.36 and 0.02 pM.

In this study, we established the conditions for an assay detecting estrogenic activity that is approximately 10 to 100 times more sensitive compared to other bioassays. In addition, our assay does not require cell lysis to measure bioactivity, thus allowing assessment without sample pre-concentration. The high sensitivity shows that the cells can be used to detect E2, EE2 and E1 activities at low levels, which are required to meet regulatory standards. Also, activity measurement without cell lysis opens the possibility of developing an effect-based-biosensor that would allow for automatized analysis. Importantly, the developed assay has an in vivo-relevant REP for EE2 of 23 compared to E2, something that the current in vitro assays for estrogenicity have failed to demonstrate. This is an advantage over other bioassays and adds to the benefits of this assay as a potential solution to meet the suggested regulatory changes for estrogens monitoring in inland waters.

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6.05.P From Pollution Hotspots to Pro-Environmental Behaviour Change: The Role of Citizen Science in Observing and Addressing Emerging Contaminants

6.05.P-Th230 Ocean Literacy and Art: How Raising Awareness through Art promotes Behaviour Change
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The Ocean sustains life on Earth through essential ecosystem services, including carbon and nutrient cycles, production of oxygen, provision of food, mineral extraction, and supply of biomolecules used in cosmetics and pharmaceutical products. Although scientists understand environmental processes and interactions, the general public often lacks awareness of fundamental Ocean dynamics, mostly due to limited engagement rather than lack of information.

To improve Ocean engagement within local communities, the Ocean Literacy (OL) education framework was applied and contextually adapted. This framework, which explores the “understanding of the Ocean's influence on us, and our influence on the Ocean”, is built on seven fundamental principles that connect Ocean knowledge with diverse disciplines, such as biology, chemistry, geology, physics, economics, and visual arts.

In this work, the OL framework was used to empower artists to become local Ocean ambassadors. A call for artists was launched within the Waves of Change project, where six artists were selected to participate in a residency designed to deepen Ocean knowledge, in a unique experience. The residence included a one-day sea expedition aboard the R/V Tom Crean, a month-long residence in Galway, a visit to the local aquarium, and four masterclasses with scientists and artists on sustainable aquaculture, parasites, noise and microplastic pollution, biodiversity, and music. These activities provided opportunities for artists to collect audio-visual materials and to interact with researchers, PhD students, vessel crew and aquarium staff.

During the residence, artists translated complex scientific concepts into artworks using diverse media such as sculpture, photography, art installation, videoart, animation, illustration, and sound. Their pieces addressed biodiversity, climate change, underwater noise, ocean acidification, and plastic pollution, were displayed in the Ripples art showcase, in March 2025. This co-designed immersive exhibition engaged 150 visitors, promoting dialogues on Ocean challenges and solutions.

This work highlights the importance of empowering artists as local Ocean ambassadors, capable of translating complex scientific concepts into art pieces that promote community dialogue, reflections and even behaviour change. Such initiatives build communities of practice and encourage dialogue and shared responsibilities over the Ocean and its resources, in a sustainable and harmonious way.

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6.05.P-Th231 A Decadal Assessment of Korea's Comprehensive Nation-Wide Marine Ecosystem Survey: Integrating Stressor Diagnostics and Ecological Health Risk Evaluation

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The National Marine Ecosystem Survey (NMES) of the Republic of Korea has been operating for a decade under a biennial cycle, covering coastal, tidal flat, rocky shore, and seabird ecosystems, and integrating physical, chemical, and biological indicators to assess ecosystem structure and function across multiple spatial and temporal scales.

Using ten years of NMES data, we developed a diagnostic framework for ecosystem health evaluation and dominant stressor identification. Indicators such as salinity, nutrients, chlorophyll a, zooplankton biomass, sediment grain size, organic carbon, and benthic macrofaunal density were analyzed through reference-condition approaches, outlier detection, and artificial-intelligence-assisted anomaly recognition. The results demonstrate distinct ecological typologies across the four Korean seas (West, South, East and Jeju)—reflecting differential patterns of nutrient enrichment, sediment stress and biological community responses.

The computed Ecosystem Health Index (EHI), based on an Ecosystem Outliers Index (EOI) framework, revealed clear spatial gradients and temporal shifts correlated with eutrophication and organic loadings. Moreover, ecosystem stress was found to be driven not only by the intensity of human activities but also by structural vulnerabilities inherent to natural systems, such as tidal effects including current velocity, direction, and tidal ranges, which amplify stress responses in specific habitats. The framework enables identification of anomalous conditions and supports assessment of cumulative multi-stressor risks.

This work contributes to ecological risk assessment by bridging large-scale monitoring and management applications. It highlights a shift from pollutant-centric monitoring to an ecosystem-based, risk-informed diagnostic system. The proposed approach strengthens the scientific basis for marine ecosystem protection, prioritized restoration and sustainable policy development.

Keywords:

marine ecosystem monitoring, ecosystem outliers index, ecosystem health index, stressor diagnostics, ecological risk assessment, long-term monitoring, Korea

6.05.P-Th232 Whats in our water? Water testing design to engage different audiences

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Water quality is of increasing concern globally, within increasing risk of water scarcity due to changes climates and extreme weather in addition to an increasing quantity and diversity of water pollution. There is a lot of public interest in this topic, from a range of different communities who are keen to explore and advocate for their local spaces. Making water testing accessible, and understandable is an important element of engaging people and empowering them to advocate for their spaces. Through a citizen science project working with young people we designed a method of water testing that could be done without any external researcher input enabling them to monitor their local environments for varying water quality and key pollutants such as nitrates and phosphates. We also tested for turbidity using a Secchi disk, water temperature, pH and conductivity using a probes and looked for potential indicators of varying water quality too (such as plastic pollution). Following on from the successful in-field work, we designed a water testing activity that can be done, really easily, in a classroom/open day/science festival where we explore the importance of building broader environmental questions into the materials to increase the confidence (and enjoyment) of the people taking part. These questions can range from how the scientific process works, through to the importance of including evidences and having a baseline of data when campaigning to the best ways to visualise results. The project was hugely enjoyable and successful, and the table top activity has been piloted at science festivals, in school and at drop in events aimed at young people. The citizen science community involved in the project have been essential to shaping this work, and we highlight through this the importance of working with communities to create the best resources and study designs for the specific groups to be able to monitor their environments effectively, and showcase their results in due course!

6.05.P-Th233 Sunscreen User Habits and Public Perceptions of Sunscreen Pollution: A Case Study from a British Coastal City

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Sunscreens are topical personal care products (PCPs) that contain ultraviolet (UV) filters to protect against UV radiation. These filters are considered chemicals of emerging concern (CECs), due to their widespread use and global distribution. Understanding public perceptions of marine pollution is essential for assessing consumer awareness and behaviour. However, existing studies largely focus on public health perspective and application rates, with limited attention to public awareness of sunscreens as marine pollutants.

This study aimed to review the current knowledge base on public perceptions of sunscreen pollution, provide insights into marine policy and regulation of sunscreen ingredients, and explore public awareness and attitudes in the UK. A literature review and critical evaluation of global regulatory developments were conducted alongside a series of public outreach events. A total of 281 participants voluntarily responded to a set of questions covering sunscreen habits, emotional responses and environmental awareness. 89% of respondents reported using sunscreen, and 81% were unfamiliar with the term 'reef-safe'. 55% had never considered sunscreens as harmful to the marine environment.

Qualitative thematic analysis revealed key public concerns, including environmental impact, demand for clearer product labelling, and calls for further research. These findings suggest a significant gap in public understanding of sunscreen-related marine pollution and underscore the importance of improved education, transparent marketing, and stronger regulatory frameworks. This study provides a foundation for future research and policy development aimed at aligning consumer behaviour with marine conservation goals. It also reflects an integrated approach to bridging science, policy and public engagement in addressing emerging environmental challenges.

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6.05.P-Th235 Collecting microfibres and behavioural insights through a citizen study on microfibre emission from household clothes washing

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Citizen science can serve the dual purpose of contributing to scientific data collection as well as to assess citizen's perceptions and behaviours with respect to a topic of interest. Here, we present the outcomes of an innovative citizen science project on synthetic microfibre release during home washing, designed in collaboration between environmental scientists and psychologists.

The release of microfibers during washing of synthetic textiles (e.g. polyester) is a major contributor to the emissions of microplastics to the environment. However, mechanisms driving microfibre emissions are poorly understood and little is known about the willingness of individuals to adopt pollution-reducing measures. Existing studies on the impact of textile properties or of washing conditions on microfibre release are limited by non-realistic laboratory set-ups or case-by-case assessments and do not include behavioral dimensions. In this citizen science project washing practices and related microfibre release were studied in two different home-washing campaigns in the Netherlands (involving 50-80 citizens each), allowing for a fine-grained data collection in real-life settings. Citizens were equipped with either washing bags or external washing machine filters (both commercially available) to collect microfibres released from their everyday clothes during typical home washing conditions over the course of several months. In addition to collecting the microfibres, participants reported data on textile characteristics and washing conditions. Alongside this data collection, psychological surveys, before and after participation, were completed by the citizen scientists to assess (changes in) perception, behaviours and pro-environmental attitudes.

This study yielded insights into total amounts and characteristics of microfibres released under different washing conditions and for different clothing items; and also revealed that participation in such a data collection impacted problem awareness and perceived responsibility, but did not significantly change personal norms, social norms about sustainability or perceived behavioural control.

6.05.P-Th236 Empowering Citizens to Monitor Beach Macro Litter: A Mediterranean Case Study Using the REMEDIES App

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Plastic pollution continues to threaten the ecological integrity of Mediterranean shorelines, where dense populations, heavy tourism, and infrastructural gaps drive ongoing litter buildup. Detecting and addressing these pollution hotspots calls for scalable monitoring methods that are both scientifically sound and socially participatory. Citizen science provides an effective route to broaden data collection while fostering awareness and pro-environmental behavior.

Within the Horizon Europe REMEDIES project, a multinational citizen-science program was launched to monitor beach macro litter (>2.5 cm) across six Mediterranean countries including Italy, Slovenia, Albania, Greece, Morocco, and France in 2024 and 2025. Volunteers participated in seasonal surveys using the REMEDIES mobile app, which offers harmonized instructions for recording the quantity, typology, material composition, and spatial distribution of litter items in accordance with the Marine Strategy Framework Directive (MSFD) guidelines.

This collaborative approach delivers high-resolution and comparable datasets across varied coastal contexts as well as strengthens public engagement with environmental issues. Early findings indicate that involvement in monitoring activities improves understanding of pollution sources and pathways among volunteers and inspires broader community participation in environmental stewardship.

Disclaimer/Disclosure:

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6.05.P-Th237 Citizen Science Monitoring of Riverine Microplastics

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Rivers play a key role in transporting macro and micro plastics from terrestrial sources to marine environments, but monitoring their occurrence is difficult. Macro plastic pollution varies strongly by location and is influenced by many parameters like littering, hydrological and shore conditions etc. which are not sufficiently investigated to date. Even less information is available for microplastics in rivers.

While volunteers have been cleaning up macroplastics from rivers for some time now— but mostly without collecting important data—there is even less awareness about microplastics in rivers. Also, scientific studies dealing with riverine microplastics mainly focus on specific large rivers in Austria and cannot provide a comprehensive picture of the pollution situation.

Plastic Pirates – Go Europe! <https://www.plastic-pirates.eu/en> is a citizen science initiative launched in Germany in 2016 and expanded across Europe in 2022 with support from the European Commission. School classes and youth groups (ages 10–16) collect and document riverine litter as well as microplastic following standardized procedures. Activities in Austria began in 2021, and first microplastic results are presented in this paper.

However, the reliability of data collected by citizen science is sometimes questioned, and particularly in the field of microplastics, where the collection methods themselves are not yet fully developed, it is unclear how useful such results can be. The aim of this study was therefore to compare the results from citizen science research with purely scientific surveys, but also to show the extent to which participation in the citizen science project Plastic Pirates influences the environmental knowledge and perception of the participants.

6.05.P-Th238 A Multi-Scale Citizen Science Framework Using Artificial Intelligence to Monitor Plastic Litter in Rivers and Streams

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Plastic pollution in and around freshwater systems remains insufficiently monitored across size classes, limiting our ability to assess environmental status and design effective mitigation measures. This work presents a flexible, citizen-science-based monitoring framework that uses smartphone imagery and Artificial Intelligence (AI) object detection to generate dense, georeferenced records of litter. Volunteers photograph debris encountered along waterways, and the images are processed through an AI model that counts and classifies items into up to 32 categories. This can enable the identification of hotspots, spatial and temporal trends, and uncommon occurrences that typically remain undocumented.

The technique serves as a natural follow-up to drone-based monitoring, extending observations to ground level and increasing resolution at minimal cost. Although applied here across Belgian rivers and streams, the approach is designed to be scalable and adaptable for use in other freshwater contexts.

To broaden detection across plastic size ranges, complementary processing flows are being developed, including approaches for identifying mesolitter, pellets, and microplastics retained on filters (approximately 300–1000 µm in this work). This multi-scale strategy supports future integration of field sampling, laboratory analysis, and automated recognition workflows.

The citizen-science model reduces logistical and financial barriers by providing a low-threshold, largely automated workflow requiring minimal expert training. While the AI system supports detailed categorisation, operational focus is placed on five prevalent litter types: cans, plastic bottles, plastic bags, cigarette butts, and cigarette packages. The resulting high-density spatial information assists partners in designing targeted interventions and informs broader environmental management strategies. Potentials, challenges, and limitations for wider implementation will be addressed.

6.05.P-Th239 Development of a tool for remote detection of plastic pellets based on relationships between eco-geomorphological characteristics and pellet concentrations along the Scheldt riverbanks, Belgium

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Since the 1970s the widespread presence of industrial plastic pellets has been observed in surface waters and beaches all over the world. Pellets are being unintentionally released into the environment along the manufacturing and logistics chain and transported far from pellet related activities. International citizen science groups are active in documenting global pellet pollution. Nevertheless, data are mainly obtained by time consuming, target-based strategies with different sampling methods, which makes it difficult to critically evaluate the results.

We present a study in the Scheldt estuary encompassing the port of Antwerp (Belgium), one of Europe's largest polymer hubs. To elucidate the relationship between the riverbanks' ecological and geomorphological characteristics and pellet concentrations, field measurements were conducted on 28 locations along the riverbanks between May 2023 and February 2025. A standardized methodology applicable in an heterogeneous landscape was developed. At each location 9 quadrats (50 by 50 cm) were placed in 3 lines, based on the deposited tidal materials. The materials within the quadrats were collected, air dried, sieved and pellets were counted. On 6 hotspots seasonal sampling took place targeting pellets, macroplastics (> 25 mm) and mesoplastics (5 – 25 mm). Drone images were obtained by using a high-resolution optical camera and a multispectral (12 band) camera on 1 location.

The results show that plastic accumulation hotspots are located close to urban (macro/meso) or industrial areas (pellets) in the river's outer bend or close to a man-made construction. Surface roughness due to the riverbanks' morphology or vegetation increases the ability to act as a plastic trap. The ratios between macro-, mesoplastics and pellets showed that the occurrence of 1 macroplastic is associated with 5 mesoplastics (median 2.0) and 31 plastic pellets (median 2.3). First drone results were promising: riverbank morphology and superficial plastics are observable. Most plastics are found within tidal deposited materials, which makes it difficult to have a realistic idea of the degree of pollution if you only look at the superficial materials.

This research is ongoing. We aim to use this knowledge and new field data to develop AI driven plastic detection tools to detect pellets by smartphone imaging or identify potential plastic pellet accumulation zones by drone imaging of the riverbanks' characteristics linked to plastic hotspots.

6.06 Impact of Armed Conflict in Terrestrial and Aquatic Environments

6.06.T-01 Framework For War-Related Marine Damage Assessment Of Ukraine's Black And Azov Sea Territorial Waters

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Russia's war of aggression against Ukraine has caused a significant yet largely unmeasured environmental crisis in the Black Sea and Azov Sea. Conflict-related debris—including unexploded ordnance (UXO), naval mines, munitions fragments, sunken ships, and explosive residues—has introduced persistent pollutants into already vulnerable marine ecosystems within Ukraine's territorial waters. The corrosion of munitions, repeated attacks on coastal and port infrastructure, and major hydrological disruptions such as the destruction of the Kakhovka Dam have collectively released fuels, metals, explosives, and toxic combustion byproducts into coastal and offshore waters. To address this threat, a marine environmental damage assessment framework has been developed that integrates two environmental liability models—the U.S. Natural Resource Damage Assessment (NRDA) and the European Union Environmental Liability Directive (ELD)—both adapted for wartime conditions and guided by the principles of International Humanitarian Law (IHL). The framework is based on an evidence-driven approach to quantify injuries and damages, aligning with US NRDA and EU ELD methodologies. We present an assessment framework for evaluating the severity and extent of environmental impacts on Ukraine's coastal and marine resources in Black Sea and Azov Sea territorial waters. The framework is a five-step approach that begins with a desktop preliminary screening analysis, comparing the pre-conflict baseline (defined as conditions before the annexation of the Crimean Peninsula) and current environmental conditions to ascertain the types and extent of service losses using available data (step 1). It then proceeds to fill critical data gaps and verify current environmental conditions (step 2), followed by a detailed damage assessment using classical economic methods (step 3). The results inform a restoration strategy (step 4) and the monitoring and adaptive management strategies needed to support long-term recovery and restoration of marine resources (step 5). It offers the foundation for a long-term marine recovery strategy that aligns Ukraine's post-war reconstruction with EU environmental standards and regional cooperation mechanisms.

6.06.T-02 Toxic Traces of Tirpitz: Using Microbial Communities to Identify Shipwreck Pollution

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Shipwrecks have a considerable impact on their surrounding environment. In the case of warships, corrosion causes the release of contaminants from both fuel and explosives, which contaminate sediments, water and marine biota. Due to their toxic and carcinogenic properties, explosives like 2,4,6-trinitrotoluene (TNT) represent a significant environmental concern. Marine organisms exhibit cellular stress responses even at low exposure levels and can bioaccumulate TNT and its metabolites, posing a potential risk to the human seafood consumer.

Despite ongoing corrosion processes and an expected increase in leakage of harmful substances, no comprehensive monitoring of wrecks is in place. Microbial communities, which are sensitive indicators of environmental changes, offer a promising and accessible solution, addressing challenges such as the complexity and diversity of pollutants. Initial studies have shown that bacterial community changes around shipwrecks can be linked to hydrocarbons or steel, however, no research explored microbial responses to explosives.

This study investigates microbial changes around the remains of the World War II battleship Tirpitz, which sank 1944 close to the city of Tromsø, Norway. Sediment and water samples were collected by divers, partly next to remaining munitions. Explosives (TNT and its main metabolites) were measured using gas-chromatography combined with mass spectrometry (GC-MS/MS). Next, microbial communities will be analyzed using 16S rRNA nanopore sequencing and related to concentrations of explosives in sediments and water.

Our results revealed TNT in sediments in all collected samples, with the highest concentrations next to a depth charge, indicating leakage into the surrounding environment. Based on our previous investigations on a marine sediment microbiome heavily influenced by explosives in a munition dumping area, we expect a sensitive shift in microbial communities exposed to higher concentrations of TNT. This work highlights the influence and potential risks of shipwrecks for their surrounding environment, and microbial community changes related to leaking explosives will help to identify taxa potentially serving as indicators for shipwreck pollution.

6.06.T-03 Warship wrecks and their munitions cargo as a threat to the marine environment and humans: the V 1302 "JOHN MAHN" from World War II

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Beside several million metric tonnes of all kinds of munitions dumped into the seas during and after the two World Wars several thousand ships containing munitions and fuels were lost at sea. The munition casings are now corroding and releasing their toxic chemicals into the environment thereby posing a risk to the marine ecosphere and the human seafood consumer.

In the course of the European Interreg research project North Sea Wrecks the "Vorpostenboot V 1302" (civilian name John Mahn) at the Belgian coast was selected as a case study to focus on munitions onboard the wreck and their impact on the marine environment.

The wreck site of the V 1302 and surrounding areas were mapped by scientific divers and a multibeam echosounder. Water and sediment samples were taken in a cross-shaped pattern around the wreck. To assess a possible entry into the marine food chain, caged blue mussels were exposed at the wreck, and fish that stays locally at the wreck were caught. All samples were analyzed for the presence of TNT and metabolites thereof by GC-MS/MS analysis.

TNT was the most abundant energetic compound (EC) in water and sediment samples, while the TNT metabolites 1,3-dinitrobenzene, 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene were less abundant. Caged mussels revealed the presence of explosive chemicals in mussels at the V 1302 wreck, with an average total concentration of 2 to 4 ng/g mussel dry weight, while passive samplers showed a mean total concentration of approximately 10 to 40 ng per sampler for all the energetic compounds.

Finally, energetic compounds were detected in fish (*Trisopterus luscus*) caught at the V 1302 wreck. In fish filets TNT was the most prominent EC, while TNT metabolites were less abundant. Surprisingly, fish bile (which serves as a major excretion route in an organism from the liver to the intestine) contained only low concentrations of TNT metabolites, while TNT itself was absent. These findings hint at an alternative route for

the entry of EC into fish.

Evidence was provided in this study that sunken warship wrecks emerge as a point source of contamination with nitroaromatic energetic compounds leaking from corroding munitions cargo still on board. Fortunately, so far, the concentrations found in mussel flesh and fish filet were only in the one-digit ng per gram range thus indicating no current concern for the human seafood consumer. However, in the future the situation may worsen as the corrosion continues.

6.06.T-04 Revisiting Deposition of Ammunition in Swiss Lakes: from Monitoring Organic Munition Constituents to Assessing their Release and Fate

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Chemical pollution of aquatic and terrestrial environments with munition constituents (MCs) such as explosives from the legacy of military activities poses a potential risk to ecosystems and human health. The exposure assessment of the diffuse pollution originating from several hundred thousand tons of ammunition dumped worldwide into aquatic environments during the 20th century was, however, impeded by the sampling resolutions, instrumental sensitivities, and/or the scope of quantified MCs of past monitoring studies. Moreover, the ongoing deterioration of ammunition casings, which is closely tied to environmental conditions, might alter the likelihood of MC emissions and is understood only scarcely. This is especially the case for landlocked countries such as Switzerland, where freshwater lakes hold not less than 14 thousand tons of ammunition.

In our study, we are therefore designing sampling procedures and analytical methods to investigate the emission and fate processes of MCs from ammunition deposited in freshwater lakes. Specifically, we are developing methods for the quantification of 26 MCs and selected transformation products in water and sediments in the low to sub- ng/L and µg/kg concentration range with liquid chromatography-high resolution tandem mass-spectrometry (LC-HRMS/MS). Current limits of quantification for water analysis, which range from 0.01 to 1 µg/L, are being improved by implementing an enrichment step using solid-phase extraction (SPE). Using an accelerated solvent extraction (ASE) method for sediment analysis, very good absolute recoveries of 80-120 % are obtained for approximately half of our analytes. To support the evaluation of MCs distribution and ammunition casings deterioration, we will perform complementary analyses including (1) Pb-210 dating, (2) X-ray fluorescence (XRF) scanning with a focus on heavy metals, and (3) clay and total organic carbon (TOC) content estimation as indicators for MC sorption to sediment constituents. These analytical procedures will be applied to samples collected over selected open-water ammunition deposition sites in Switzerland. To increase sampling resolution, we successfully tested the deployment of a multicorer which allowed to simultaneously retrieve 6 cores of 60-80 cm at fixed relative horizontal distances at 215 m underwater in 20 min. This study will provide valuable information for the potential needs and risks of recovering ammunition lying at the bottom of Swiss lakes.

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6.06.P Impact of Armed Conflict in Terrestrial and Aquatic Environments

6.06.P-We416 Biological Assessment of Military Contamination in the Oceans

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After World War II, enormous quantities of weapons were sunk in the oceans. Chemical weapons are not expected to have any long-term effects on the environment, as they react extremely quickly under water with all biological materials. The extent to which this is scientifically sound has yet to be clarified. However, it is also clear that no access is granted to the chemical weapons that are still at the bottom of the sea. In addition, most public research on other weapons such as bombs and ammunition is excluded for security reasons. Public interest is currently focused on the recovery of old ammunition. Large sums of money have been made available for this purpose. However, the question arises as to what happens during recovery. Contaminated sediments in particular could be further distributed and damage ecosystems. However, it is clear that recovery seems unavoidable given the enormous pollution. In any case, we have attempted to further assess the environmental risks in this difficult field by examining ammunition and testing fireworks for their toxicity to

marine organisms.

We thus present an approximation of the risks to be expected from military contamination in the oceans. However, a biological assessment and accompanying biological monitoring during the salvage operations are essential.

6.06.P-We417 Dissolution Experiments on Solid Explosive Material Recovered From Sea-Dumped Munitions

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Military activities and post-war dumping have introduced large quantities of high explosives such as TNT, RDX, and HMX into terrestrial and marine environments. Residues from these explosives can persist in surface soils and dissolve into groundwater, posing long-term environmental risks. Millions of tons of munitions were historically dumped at sea, and breaching by corrosion allows dissolution and spread of explosive chemicals into the surrounding environment. Upon release, toxic explosive chemicals pose risks to marine ecosystems, seafood safety, and human health. Understanding dissolution and transport processes is therefore essential for understanding the potential exposure of munition chemicals to marine ecosystems. However, little is known about the primary controls on dissolution of solid explosives or their rates in marine systems.

The current study tested the effect of water current speeds on dissolution of explosive chemicals from fragments recovered from WWII-era munitions in the Baltic Sea. Experiments were conducted using deionised water at room temperature, simulating shaking, stirring, and flume conditions. Dissolution kinetics were assessed after solid-phase extraction and HPLC-APCI-MS analysis. Contrary to expectations, results showed limited dependency of water current speed on dissolution rate. However, during the simulated shaking conditions, the highest flow velocity (120 rpm) led to a clear increase in dissolved TNT after 24 hours. Overall, TNT levels were between 115 % and 185 % higher than at 30 rpm, approximately twice as high, indicating that stronger water movement substantially enhances dissolution. In addition, some fragments contained the explosive tetryl, enabling assessment of its dissolution behaviour alongside the more commonly detected TNT and RDX. Tetryl was detected in solution after 24 hours of exposure, indicating that it actively dissolves under the tested experimental conditions.

These results provide new insights into the dissolution behaviour of sea-dumped explosive material and provide quantitative information on the environmental release of explosive chemicals.

6.06.P-We418 Effects of Clearance Operations on the Temporal and Spatial Dynamics of Munition Compounds in Lübeck Bay, Baltic Sea.

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An estimated 1.6 million tons of munitions rest on the seafloor in the German territories of North and Baltic Sea due to dumping of bombs, torpedoes, and other munitions after World War I and II, as well as unexploded ordnance (UXO). These munitions and their associated munition compounds (MC) pose potential risks to marine ecosystems, blue economy and human health globally. Advanced degradation and corrosion of munition shells allow release of toxic explosive chemicals into the surrounding environment. In the Baltic Sea, where water exchange is limited, such contaminants can accumulate more rapidly than in open marine systems. As corrosion progresses, munition shells also become increasingly fragile, underscoring the need for clearance operations before corroded munitions become unsafe to handle.

To address these challenges, the German government has launched a pilot project for the removal of sea-dumped munitions in Lübeck Bay. The project aims to gain knowledge on clearance of various munition types, and to demonstrate that systematic clearance is feasible. The results of these clearance tests will be used further to develop a mobile platform for the large-scale, on-site destruction of recovered munitions.

Here, marine clearance operations were monitored for the first time to evaluate environmental effects. Water samples were collected during research cruises before, during and after clearance activities at four known dumping sites in Lübeck Bay. Dissolved explosives (TNT, 2-Amino-4,6-Dinitrotoluene (ADNT), RDX, HMX and Dinitrobenzene (DNB)) were preconcentrated by solid phase extraction and analysed using HPLC-APCI-MS. Before clearance, total MC concentrations ranged from 4 to 344 ng/L. Preliminary results show a reduction after clearance at only one of the sites. Where munition piles were only partially cleared, the decrease in concentrations was insignificant. Furthermore, the clearance changed the munition compound composition, with DNB, RDX, and TNT dominating before, and ADNT and TNT afterwards, suggesting ongoing degradation processes. RDX showed the highest pre-clearance concentration on average (18 ng/L), but was below the detection limit post-clearance, indicating a successful removal. Samples collected every eight hours during clearance showed strong fluctuations, but no increase in overall concentrations. These findings provide important insights into the immediate effects of marine munitions clearance operations and their efficiency.

6.06.P-We419 Environmental Impact of WWII Warship Wrecks and Munitions in the English Channel: Evidence of Long-Term Contaminant Release

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Historic shipwrecks are increasingly acknowledged as significant sources of marine pollution. Many carried substantial amounts of fuel, paint, electrical systems, and munitions that can release toxic substances as they corrode. However, the data needed for a reliable and accurate large-scale assessment of their environmental impact remain scarce. The CONTEPAV project investigates the environmental impact of such wrecks, focusing on contaminant release processes and spatial patterns in the eastern English Channel, a highly dynamic environment with strong hydrodynamics and high wreck density.

Four representative warship wrecks were studied, including the British minesweeper HMS Magic and the German vessels Greif and Gauss. Surface, inside the wrecks, and core sediments were collected at varying distances, complemented by passive samplers (SPMD, DGT, POCIS) deployed on the wrecks to detect ongoing pollutant releases. Sediment cores were divided to 3 sections of about 14-15cm, corresponding to bottom, medium and top core sections. Analyses focused on hydrocarbons (PAHs, alkanes), metals, PCBs, and explosive compounds (TNT and breakdown products).

Results reveal decreasing vertical metal concentrations toward the top core section, consistent with a slowing in metal releases from the shipwrecks. Copper and zinc show significant enrichment near wreck structures, exceeding both ERL ecotoxicological thresholds. Correlation between distribution patterns suggest that manganese and vanadium concentration are not influenced by the structure. Many samples contain high PCB concentration above and EAC thresholds for several congeners (PCB 118 dominates the PCB profile). Highest PCB concentrations are found in sediment collected inside the wrecks, confirming a direct link with onboard PCB containing materials. Although TNT concentrations in sediments were below detection limits, passive samplers detected nanogram-per-liter releases, confirming ongoing but low-level leakage from corroded munitions.

These findings demonstrate that WWII wrecks continue to act as localized and persistent sources of contamination, decades after sinking. The results highlight the need for long-term environmental monitoring and integrated risk assessment frameworks to address underwater cultural heritage and war remnants as potential pollution sources.

6.06.P-We420 Hotspots of Environmental Contamination from Explosive Chemicals Released from Sea-Dumped Munitions

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The German Baltic Sea is widely contaminated with low levels of dissolved explosive chemicals (or munition compounds, MC), including 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitro-1,3,5-triazinane (RDX), and 1,3-dinitrobenzene (DNB). Chemical mapping in the water column has shown clear spatial differences among different explosive compounds, with TNT concentrations especially high in Kiel Bay, and RDX and DNB concentrations relatively elevated in Lübeck Bay. The reason for these spatial trends is not known, but has been hypothesized to result from varying explosive mixtures in different munitions types dumped in the two regions. In the current study, water samples were collected throughout the study region with a Niskin rosette, and close to munition objects using a small ROV and a towed video platform. Additional water samples were collected at the seafloor with a lander that performed automated solid-phase preconcentration. Dissolved MC were analyzed immediately after sample collection by shipboard automated solid-phase extraction and analysis by HPLC-APCI-MS. Deep water samples showed the typical pattern of high TNT concentrations in Kiel Bay (up to 10 ng/L), and elevated RDX and DNB in Lübeck Bay (up to 203 and 137 ng/L, respectively). Targeted water samples close to munition objects showed sources of TNT, RDX, and DNB were present at all munition sites and chemical release occurred only from objects with breached housings. However, a diverse array of munitions targeted in Lübeck Bay revealed clear differences among objects, with sources emitting either 1) all three MCs but predominantly TNT, 2) moderate TNT and high RDX and DNB, or 3) high DNB and low TNT and RDX. The toxicity of these MCs to aquatic organisms follows the order DNB > TNT > RDX. Thus, the environmental risk posed by different munition types would follow the same order (all else being equal), with objects containing the Type 2 (high DNB/RDX, moderate TNT) or Type 3 (high DNB, low TNT/RDX) explosives posing the greatest environmental health threat. For environmental protection, the highest priority for remediating munition objects should focus on breached objects with DNB-containing explosive fill. The current study shows that regional mapping can identify high-risk regions. A combination of geophysical target mapping,

visual object inspection, and targeted water sample analysis then provide data for definitive prioritization of munition objects for remediation.

6.07.A Innovative Strategies in Implementing Safe and Sustainable by Design (SSbD) Approaches

6.07.A.T-01 Developing a Safe and Sustainable by Design (SSbD) Bio-based Chemical Platform within the NEXT-STEP Project: Focus on Hazard and Worker Exposure Assessment (SSbD Steps 1 & 2)

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The European NEXT-STEP project (1 June 2024 – 31 May 2028), funded by the Circular Bio-based Europe Joint Undertaking (CBE-JU), aims to demonstrate a sustainable, safe, and economically viable production process for the bio-based chemical platform 3MdVL and its derivative 3MPD. These monomers are designed to replace fossil-based polyols and monomers, with applications in PLA co-polymers and recyclable NIPU foams. Ensuring safety during development, NEXT-STEP implements a Safe and Sustainable by Design (SSbD) framework aligned with the European Commission's Joint Research Centre methodology.

This study focuses on Steps 1 and 2 of SSbD: hazard assessment and human health and safety in production. Step 1 identifies intrinsic hazards to human health, the environment, and physical safety, using bibliographic data, MSDS, and CLP criteria. Step 2 evaluates worker exposure across laboratory to demonstration scales, using modeling tools (Stoffenmanager®, IHMod®) and, where necessary, analytical verification following international standards (EN482:2021, ISO16017-1/2:2020, EN689:2018). The Risk Characterisation Ratio (RCR) provides a quantitative measure to flag potentially unacceptable exposures, guiding mitigation or substitution strategies.

Preliminary results indicate that most chemical building blocks pose low risk, while a few NIPU production substances were identified as higher risk for human health. These findings are communicated to partners to identify safer alternatives or implement mitigation measures to ensure controlled worker exposure. The iterative assessment throughout the project timeline allows integration of evolving process conditions and scientific developments.

By embedding safety considerations into early molecular and process design, NEXT-STEP demonstrates how SSbD can reduce chemical hazards, optimize production processes, and ensure inherent safety for workers. This approach enables the development of 3MdVL and 3MPD monomers that meet functional performance targets while minimizing environmental and human health risks, illustrating a practical pathway for sustainable bio-based chemical innovation.

Keywords: Safe and Sustainable by Design, bio-based chemicals, worker exposure, hazard assessment, 3MdVL, 3MPD

6.07.A.T-02 A Tiered Ecotoxicity and Biodegradability Testing Approach of Novel SURFsUP Biosurfactants for Home and Personal Care Products and Agrochemicals

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The SURFsUP project aims to develop next-generation biosurfactants that are safe, sustainable, and biodegradable for applications in home care, personal care, and agrochemicals. To ensure the implementation of the Safe and Sustainable by Design (SSbD) Framework established by the European Commission-Joint Research Centre, a comprehensive experimental testing approach was implemented to evaluate environmental safety and biodegradability of the biosurfactant formulations.

A stepwise approach was adopted, starting with acute ecotoxicity tests on aquatic and soil organisms from different trophic levels including OECD 201 (algal growth inhibition -R. subcapitata), OECD 202 (Daphnia magna immobilization), and ISO 16072 (soil respiration -bacteria). Additional tests included OPPTS 850.420 (seed germination/root elongation -L. sativa-) to broaden species coverage. The second step comprises chronic tests such as OECD 208 (seedling emergence and growth -C. sativus-), OECD 211 (Daphnia magna reproduction) and OECD 220 (earthworm reproduction -Enchytraeide crypticus-). Biodegradability was assessed using OECD 301F standard test (manometric respirometry) and a rapid MTT screening assay to confirm microbial viability and degradation potential.

Preliminary results show low (or none) acute toxicity of the biosurfactants across the tested species, with EC50 values higher than 100 mg/L in all cases, confirming their suitability to be tested in chronic tests.

Biodegradation in soil and water compartments will be tested to investigate their potential persistency in environment once the biosurfactants formulations are released in wastewater treatment plants during use and end of life stages.

The integration of ecotoxicological investigations and biodegradation assays through a tiered approach helped to reduce experimental testing and prioritize the most promising biosurfactant formulations to support the SSbD implementation for home and personal care products and agrochemicals.

Disclaimer/Disclosure:

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6.07.A.T-03 DECISION T-REEX: A Decision Tree for Environmental Exposure Assessment for (Advanced) Materials

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Estimating environmental exposure during early product development stages is often limited by a lack of detailed input data and accessible methods. Existing exposure assessment tools, while well established, are often too complex for early-stage decision-making for (advanced) materials and offer limited flexibility under uncertainty.

To address this gap, Decision T-REEX was developed as a modular tool that estimates emissions to air, water, and soil across the life cycle of (advanced) materials. The tool guides users through structured questions covering production, manufacturing, use and end-of-life stages. The use phase are further divided into service life, application before use and maintenance, allowing the tool to capture diverse release pathways. Based on the provided information, Decision T-REEX assigns release scenarios using Environmental Release Categories (ERC) defined by the European Chemicals Agency (ECHA) and, where applicable, Specific Environmental Release Categories (SPERC) developed by recognised industrial sector organisations. On top of that, it includes a feature to flag scenarios of potential concern and suggests mitigation actions.

The tool was tested on three industrial case studies: a sunscreen with nanosized titanium dioxide, a post-harvest fruit coating, and an anti-corrosion coating. For each case, life-cycle specific emission estimates to environmental compartments were produced. For the sunscreen and fruit coating, the tool identified complete release to water during use. The anti-corrosion coating, while applied indoors with minimal release, was flagged for potential environmental exposure due to moderate degradation in outdoor settings.

Decision T-REEX showed promising performance in clarifying where releases may occur across the life cycle and how design or use-phase conditions could influence them. Even when only limited information is available, it supports early-stage decision-making by offering a transparent and structured basis for exposure assessment. The tool will be integrated into the digital platform of the European-funded SUNRISE project, which aims to advance the practical implementation of Safe and Sustainable by Design (SSbD) methodologies for advanced materials.

Disclaimer/Disclosure:

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6.07.A.T-04 A Multilevel Qualitative Assessment Tool for Safety Screen-ing of Advanced Materials

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The JRC Safe and Sustainable by Design (SSbD) framework for chemicals and materials promotes the development of safe and sustainable chemicals/materials from the early design phase. In this context, the Horizon Europe SUNRISE project has developed a questionnaire-based tool—building upon a previous version from the H2020 SUNSHINE project—to support the early-stage safety assessment of advanced materials (AdMa) synthesized for innovative applications. The tool helps companies to identify potential human health and environmental risks along the AdMa life cycle, already in the first stages of material development. The questionnaire follows a multilevel structure, where top-level questions address the most critical safety aspects across the AdMa lifecycle and guide users to specific sub-questions only when needed. Another advancement is the integration of suggestions for suitable tools and analytical methods that can be used to address data gaps identified during the assessment. To evaluate its applicability, the tool was tested on an industrial case study within the SUN-RISE project. The case study involved TiO₂-based AdMa, obtained through different synthetic processes, intended for use as UV filters for sunscreen formulations. The process enabled the identification of key life cycle stages (synthesis and use phase) and exposure routes to be considered in the assessment. Thus, it supports the identification of data needs and knowledge gaps that must be addressed to align with evolving regulatory requirements and to facilitate the design of safe and sustainable AdMa.

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6.07.A.T-05 Retrospective SSbD case study of a small-molecule antibiotic: cross-domain evaluation of safety and sustainability in pharmaceutical development

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The pharmaceutical sector is essential for human health and wellbeing but is associated with high environmental and occupational burdens, especially for small-molecule medicines with long, solvent-intensive syntheses. Applying Safe and Sustainable by Design (SSbD) principles to pharmaceuticals is challenging because development can last 10–15 years, and the information needed for safety and sustainability assessment is often fragmented and unavailable when key design decisions are taken.

We conducted a retrospective SSbD analysis of a recently developed antibiotic, in collaboration with a F. Hoffman-La Roche AG. The aim was to understand how safety and sustainability considerations could have been integrated earlier in the research and development pipeline. We first reconstructed the “data landscape” of the molecule: for each development phase, from early discovery to late-stage development, we identified which data are generated, by whom, in which format (qualitative, quantitative, predicted or experimental), and whether they are usable for safety and sustainability assessment.

We then performed an ex-ante life cycle assessment (LCA) of the manufacturing route, guided by the forthcoming PAS 2090 standard on life cycle assessment of pharmaceutical products. Because existing LCA databases contain very few pharmaceutical intermediates, reagents and specialised solvents, we developed a structured proxy approach. Proxy chemicals were selected using structural and functional similarity and information from the reaction database Reaxys, so that key energy and material hotspots could still be screened while keeping assumptions transparent.

In parallel, we explored how early molecular and process information can support environmental risk assessment (ERA) and worker protection. Using simple descriptors derived from chemical structure and minimal route knowledge (for example partition coefficients, acid dissociation constants, functional groups, structural alerts, etc.), combined with tools from the Partnership for the Assessment of Risks from Chemicals (PARC) Toolbox, we tested how early signals of persistence, mobility and occupational hazard could have been identified.

Our work proposes concrete insertion points for SSbD in pharmaceutical innovation and outlines minimal data

packages and proxy rules that can enable simplified, cost-effective safety and sustainability assessment at early stages, supporting more sustainable antibiotic development.

Disclaimer/Disclosure:

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6.07.B Innovative Strategies in Implementing Safe and Sustainable by Design (SSbD) Approaches

6.07.B.T-01 ProScale Service Life: Assessing Direct Use-Phase Exposure in Early Innovation

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Introduction

Applying the Safe and Sustainable by Design (SSbD) framework requires tools that capture toxicological impacts of chemicals from a life cycle perspective to guide innovation [1]. Due to the lack of tools, direct impacts on consumers during the service life of products has been hard to capture. ProScale is a risk-based, life cycle-oriented approach for chemical footprinting that was developed to address this gap by estimating toxicity potentials across the life cycle of products [2]. Therefore, the ProScale method is applicable in the context of the SSbD as a bridge between risk assessment and life cycle assessment (LCA). Herein, we present the recently developed ProScale for Service Life (ProScale-SL) module for assessment of consumer impacts during the service life of products.

Methods

The ProScale-SL module assesses toxicological impacts on consumers in various types of indoor environments. The SL module is, as seen Eq. 1, based on five factors, of which the first four correspond to the characterization factor (CF):

- $PS_{i,r}$ (mg): route-specific process score for substance i ,
- m_{ref} (kg): reference-flow mass for one product unit,
- $PPDD_{i,r}$ (mg/person/day): per-person daily systemic dose for exposure to substance i and via route r ,
- $PHFH$ (person-days): person-time factor over the functional unit horizon, aggregating exposure across all users within this time horizon in an archetypal indoor environment (AIE),
- HFi,r : hazard factor, primarily based on hazard phrases from the Globally Harmonised System,
- MF (kg): inventory mass flow of products in the modelled system.

$$ProScale = \sum_{Substances} ((HF \times PPDD \times PHF) / m_{ref}) \times MF \quad (Eq.1)$$

Results and Conclusions

Here we revisit a fictive case study where e.g. Bisphenol A based polycarbonate was assessed with the ProScale for Workers methodology. We apply the SL module to the bottles in a household AIE. Preliminary results indicate that the module can capture the impacts of hazardous substances.

In summary, we see that the ProScale-SL module can cover the impacts of concerning substances which may be harmful to humans in the service life phase. With this module, integrated alongside already existing ProScale modules, and new developments, we foresee that the ProScale methodology will become a tool suitable for LCA practitioners, risk assessors, and others concerned with the impacts of chemicals across the life cycle of products.

Presentation preference: Platform presentation

6.07.B.T-02 How to consider benefits related to functionality in SSbD? An overview of early phase benefit assessments and an application to selected case studies

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Even though the Safe and Sustainable by Design (SSbD) framework considers many safety and sustainability aspects from the innovation and design phase to the fully developed materials and products, there is still a gap in the consideration of functionality-related benefits of chemicals or materials. To address this gap, we propose the implementation of an early-phase benefit assessment that considers functionality-related benefits alongside societal impacts; accounting for both positive and negative aspects and applied it to two cases: a wound care solutions used in healthcare and antimicrobial coatings based on upconverting particles used in public spaces. First, specific indicators for both positive and negative aspects were defined, and a qualitative scoring system was assigned. Negative indicators include the development of antimicrobial and cross-resistance, as well as animal testing for both cases. Positive indicators include reduced infections for both cases; additionally, for wound dressings, improvements in wound healing and shorter hospital stays were considered, while for antimicrobial coatings, increased public health due to reduced chemical usage and improved quality of life were included. A balance factor was calculated by dividing the sum of points from positive indicators by the sum of points from negative indicators. The balance factor was found to be 2 for wound dressings, and between 1-2 for upconverting particles, indicating that potential benefits are likely based on the functionality. In addition, guidance is provided on extending the assessment from qualitative to semi-quantitative and quantitative methods as the TRL level develops.

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6.07.B.T-03 Implementing SSbD in battery recycling processes of RESTORE and potential applications of SSbD data for the EU Battery Product Passport

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The project RESTORE aims at developing innovative, safe, cost effective and scalable solution for the separation, recovery, purification, upcycling, and validation of materials and metals, to be directly transformed into new battery precursor and functional materials. In this context, the SSbD framework developed by the EU Joint Research Centre is implemented to ensure the integration of the SSbD principles and assessment, that will result in safer technologies with improved recovery yield and increased quality of the recycled/recovered materials. Finally, it will provide a demonstration on how data and information collected for the SSbD assessment can be embedded in the EU battery product passport, to actively support decision-making at all stages of the battery recycling step, from material valorisation to recycling-process safety and second-life applications.

The SSbD assessment is being performed by collecting data and information in specific templates and by using predictive models or experiments/ measurements. The SSbD assessment results obtained are shown and discussed with interested partners (actors in the recycling steps/ processes) to make them aware of possible hazards coming from materials generated during pre-treatment processes, or in relation to release and exposure issues specific to the pre-process assessed. This allows defining corrective measures or to re-design specific steps of battery pre-processing steps. Finally, we will demonstrate how the data and information retrieved along the SSbD assessment can be included in the battery product passport, to provide to the different actors in the battery value chain key information on safety and sustainability of materials and processes.

SSbD principles will be applied and monitored to improve different aspects of safety and sustainability of battery pre-treatment processes simultaneously, with the aim to ensure resource-efficient production, addressing adverse effects on humans and ecosystems.

In a SSbD perspective, including key SSbD data to the battery product passport will empower end-users of battery materials/parts facilitating their decision-making (e.g. to decide on safer materials or processes or providing safer working conditions to protect workers health). Furthermore, the SSbD data will add value to the information reported in the battery product passport, allowing to anticipate safety and sustainability issues as well as anticipating future legal requirements.

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6.07.B.T-04 Operationalising Safe and Sustainable by Design for Industry: Mapping, Coordinating, and Structuring Chemical and Material Innovation with the PLANETS Workflow

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The Joint Research Centre has accelerated the transition towards safer and more sustainable innovation in the EU through the introduction of the Safe and Sustainable by Design (SSbD) framework. To support its operationalisation, several tools and initiatives have emerged, including CEFIC's SSbD approach, the PARC toolbox, and IRISS project. Among these efforts, EU PLANETS project addresses 11 key challenges to SSbD operationalisation—such as integration of safety and sustainability considerations, embedding SSbD within innovation processes, and improving data management. By developing a tiered, multi-criteria workflow grounded in SSbD principles, PLANETS aims to promote the design for safer alternatives for plasticizers, flame retardants, and surfactants. This contribution presents the PLANETS workflow and early insights from three case studies (CS).

The workflow was co-developed by a multidisciplinary team through iterative cycles, tackling key issues. Drawing on insights from the literature, the resulting structure includes two main phases—scoping and assessment—supported by an educational kit. The assessment phase is further subdivided into simplified, intermediate, and full tiers, enabling a progressive and adaptable evaluation process.

The scoping phase covers the motivation for (re)design, value chain definition, regulatory landscape analysis and SSbD specification. These steps help clarifying objectives, system boundaries, and resource needs at an early stage. In the assessment phase, defining core product functions remains essential. The overall process requires adapting to varying numbers of alternatives, potentially clustering similar options for feasibility. SSbD ambassadors to each CS supported coordination, stakeholder engagement facilitation, and integration of safety and sustainability from the start. Training and onboarding workshops fostered a shared understanding and reinforced collaboration, while the framework enabled multidisciplinary engagement and transparent trade-off analysis.

Overall, the workflow offers a structured and practical guide for SSbD framework implementation. Its initial application within PLANETS project demonstrates its potential value, with future refinements planned based on user feedback, expanded datasets, and broader validation across industrial contexts.

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6.07.B.T-05 Policy Gaps for Advanced Materials: Insights from EU Regulatory Mapping and Industrial Case Studies

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Advanced Materials (AdMa) support EU sustainability goals, yet their complex behaviours and life-cycle transformations challenge regulatory frameworks designed for conventional chemicals. The SUNRISE project combined a top-down mapping of EU strategies and regulations with bottom-up insights from three industrial case studies to identify gaps affecting the safe and sustainable development of AdMa and to provide evidence for future SSbD-oriented approaches.

Mapping covered 18 strategies, directives and policy instruments linked to the European Green Deal, the Chemical Strategy for Sustainability and sectoral and environmental legislation. Each instrument was reviewed for nanoform-specific data requirements, transformation and degradation considerations, release assessment provisions and coverage of life-cycle stages. In parallel, interviews with industrial partners captured challenges related to characterisation, regulatory compliance, testing needs, monitoring requirements and end-of-life handling. Findings were integrated using a harmonised framework that compared policy expectations with industrial realities.

Four cross-cutting gaps emerged. First, harmonised consumer-use labelling for nanomaterials lacks across EU product legislation. Second, nanoform-specific occupational exposure limits are absent. Third, guidance for assessing transformation, degradation and release under realistic operational conditions is insufficient. Fourth, most regulations do not explicitly address aged or weathered forms of AdMa, despite their relevance for environmental fate and hazard. These gaps were observed across different instruments revealing that many policies still assume static material behaviour.

Industrial case studies confirmed these issues. Companies reported difficulties assessing release and ageing of nano-enabled coatings under offshore stressors, limited guidance for mixture toxicity and end-of-life considerations for biocidal systems, and uncertainties when registering hybrid nano-composites under REACH. They also highlighted missing protocols for monitoring nanoparticle emissions into wastewater and the fragmentation of regulatory pathways.

Overall, the analysis shows that the EU regulatory landscape is only partially equipped to address the dynamic properties of AdMa. Addressing these gaps will require coordinated policy development, improved test methods, strengthened monitoring capabilities and harmonised frameworks aligned with SSbD principles.

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6.07.P Innovative Strategies in Implementing Safe and Sustainable by Design (SSbD) Approaches

6.07.P-Th240 Safe and Sustainable by Design: Driving Innovation Toward Safer and More Sustainable Chemicals, Materials, Processes and Products

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The Safe and Sustainable by Design (SSbD) framework, developed by the European Commission's Joint Research Centre, is described here. It is applicable to chemicals and materials, and the European Commission recommends its use in research and innovation activities. This framework is one means to facilitate achieving the goals of the European Union's Chemicals Strategy for Sustainability. The implementation of the SSbD framework calls for a new way of thinking and a shift in current innovation practices. It thus requires safety and sustainability to be considered in an integrative manner from the early stages of innovation, and with a life cycle perspective.

This work describes the policy context in which SSbD was developed, outlines the concepts underpinning SSbD, and its assessment steps. Furthermore, it presents some early implementation lessons learned, gives an overview of ongoing work to implement and advance its development, and provides an outlook for future developments. The transition to SSbD requires deeper integration of several disciplines, until now often independently practiced, that address chemical risk and sustainability. In addition, the implementation of these disciplines, from the early design phase of new chemicals, materials, processes, and products onward, makes the engagement of communities in the research and innovation fields paramount

6.07.P-Th241 Unveiling the 2025 SSbD framework

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The 2025 Safe and Sustainable by Design (SSbD) framework, developed by the European Commission Joint Research Centre (EC-JRC), builds on the foundation set in 2022 to enhance safety and sustainability across the life cycle of chemicals and materials. This revision emerged from extensive testing and stakeholder collaboration, focusing on refining the framework to better assist the innovation process. The SSbD framework integrates a co-creation process illustrated through various testing phases and workshops, aimed at gathering input for its refinement.

Central to the framework is its set of principles, designed to clarify and guide its application. The scoping analysis contextualizes key issues related to innovation, engaging actors throughout the lifecycle and proposing methodological criteria for adherence to SSbD principles. The framework introduces a comprehensive assessment structure: safety, environmental sustainability, and socio-economic sustainability. The safety assessment incorporates intrinsic chemical/material properties alongside exposure evaluations providing a holistic safety approach. Environmental sustainability employs Life Cycle Assessment to cover the chemical/material life cycle from raw materials to end of life, offering screening level assessments for less mature innovations. Socio-economic sustainability expands its focus on social fairness, competitiveness, and supply chain vulnerabilities, considering risk governance and financial stability.

The framework provides sub-chapters to evaluate safety and sustainability of processes. Additionally, the evaluation segment identifies trade-offs and uncertainties inherent in the implementation of SSbD, using dashboards for visual representation to highlight hotspots and guide innovation. A dedicated documentation chapter ensures transparent and systematic recording of the framework's implementation, fostering accountability and clarity in its application. Overall, the 2025 SSbD framework strives to guide and support innovation by addressing safety and sustainability comprehensively, supported by stakeholder feedback and robust methodological guidance.

6.07.P-Th242 A Common Language for Safe and Sustainable by Design: One Wordbook to Understand Them All

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Creating a common and shared understanding of key concepts is a critical step toward the successful operationalisation of the Safe and Sustainable by Design (SSbD) approach. As highlighted in several publications, terminology consistency remains a major challenge for the SSbD community. To address this need, a dedicated Wordbook has been developed within the PLANETs project to compile, harmonise and clarify essential terms used across SSbD-related disciplines.

This Wordbook is a carefully curated collection of more than eighty key terms and their definitions, covering domains such as safety (hazard and exposure), sustainability (environmental, economic and social aspects) including methodological tools, indicators, and frameworks relevant to the SSbD approach. The distribution of terms is well balanced between the two foundational pillars of SSbD, with 36 terms related to sustainability and 33 to safety, while generic and regulatory aspects are represented by more than 15 additional terms. Each term is classified by theme, topic, and name, providing a user-friendly structure that supports both expert and non-expert understanding.

The main objective of this resource is to foster a shared vocabulary among SSbD experts and broader innovation community. By ensuring conceptual clarity and coherence, it strengthens communication, facilitates interdisciplinary collaboration, and supports the integration of SSbD principles across research and innovation processes.

The Wordbook is made available internally as a project deliverable and publicly through the open-access platform ZENODO (and will be updated every six months), ensuring transparency and broad accessibility, as well as on the project website. It also forms part of a wider educational kit designed to build capacity, promote

training, and support the adoption of the SSbD approach across scientific, industrial, and policy communities. Ultimately, this initiative contributes to the development of a consistent and operational language that underpins the implementation of the SSbD approach across these diverse contexts.

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6.07.P-Th244 Methods and Tools for the Safety Assessment of Advanced Materials

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Advanced materials raise significant challenges for environmental and human safety assessment due to their specific physicochemical properties and behaviour across biological and environmental systems. These characteristics can limit the applicability of testing strategies originally developed for conventional chemicals, and they create a need for approaches that capture material specific mechanisms, transformations and interactions under realistic conditions.

To support this need, an extensive landscape of relevant methodologies and tools was examined and structured. Hazard identification approaches were analysed across in silico, in vitro and in vivo methods, with particular attention to techniques that address nanomaterial specific modes of action in freshwater, seawater and soil systems. In addition, computational tools for exposure, fate and risk assessment were classified into three tiers according to their data requirements, mechanistic resolution and suitability for different innovation stages. Their accessibility was also evaluated, including technology readiness level, maintenance status, cost and ease of use, providing insight into their practical applicability in both research and industrial contexts. Moreover, databases and digital tools repositories containing physicochemical information, hazard data, exposure indicators and environmental fate information were mapped to improve their usability for assessing advanced materials.

This work aligns with the Safe and Sustainable by Design framework of the European Commission by offering a structured basis for integrating safety considerations from the earliest phases of advanced materials development. Through the identification of methodological strengths, current gaps and future opportunities, the assessment landscape is clarified and strengthened, supporting responsible and sustainable innovation in advanced materials.

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6.07.P-Th245 New approach methodologies for the safety assessment of Advanced Materials

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New Approach Methodologies (NAMs) are crucial for tiers 1/2/3 Safe and Sustainable by Design (SSbD) assessment by providing a tiered, data-driven approach to evaluate chemical and material safety and sustainability. In later tiers (2 and 3), which involve more complex and data-intensive assessments, NAMs move beyond early-stage qualitative analysis towards semi-quantitative and quantitative data based integrate in vitro methods, in silico tools, and other advanced techniques. In this work, we show examples of the implementation of Tier 2/3 models for two routes of exposure (ingestion and inhalation) and methods to evaluate endocrine disruption and neurotoxicity of Advanced Materials (AdMa). Models for ingestion and inhalation (gastrointestinal triculture models and MucilAir, respectively) are used for both acute (24 hours) and subacute exposures (up to 28 days). These advanced in vitro models retain physiologically relevant exposure scenarios particularly appropriate for ingestion and inhalation, which have the potential to improve the in vivo predictability and lead to reliable outcomes. The neurotoxicity model offers a very versatile and sensitive tool for screening of neurotoxicant AdMas. For endocrine disruption, the OECD TG 455 was adapted and used to evaluate the toxicity mixtures. All exposure protocols were adapted for AdMa exposure and showed good sensitivity and great robustness in comparison to traditional monoculture models used for the same purpose. This work was supported by the EU Horizon Projects “Safe and Sustainable by Design Advanced Materials” (SUNRISE) under Grant Agreement no. 101137324, “Integrated assessment and Advanced Characterisation of Neuro-Nanotoxicity” (iCare), under Grant Agreement no. 101092971 and the Ministerio de Ciencia e Innovación, Agencia Estatal de Investigación and by Fondo Europeo de Desarrollo Regional (FEDER). Ref.No. PID2021-124729NB-I00 “MIGRABIOQUANT” (MCIN/AEI/10.13039/501100011033/FEDER, UE).

6.07.P-Th246 In vitro Ecotoxicity Models for Hazard Screening of Advanced Materials in support of SSbD Assessment

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Advanced Materials (AdMas) are increasingly being used in a wide range technological applications thanks to their unique physico-chemical properties. However, as a result of their novelty, their effect over the environment remains unknown and ecotoxicity models for hazard screening of AdMas are currently scarce. In this study, we applied two in vitro fish-based ecotoxicological models to support SSbD assessment of AdMas: a monotypic culture model using tilapia OmB brain cells and a monotypic culture model using rainbow trout RTgill-W1 gill cells. A dispersion protocol was developed using natural organic matter (NOM) to simulate environmental conditions and ensure that AdMas remained suspended during exposure. Once established, the in vitro models were exposed for 24 hours to different concentrations of AdMa suspensions (from environmentally relevant to high concentrations) and cell viability, ROS production and inflammation were assessed. Results showed that both models represent sensitive New Alternative Methodologies (NAMs) to evaluate acute effects of AdMas in this study. In both models, most of the AdMas caused effects at the highest concentrations tested over the three cell viability parameters (plasma membrane integrity, metabolic activity and lysosomal membrane integrity), but overall, ROS production was observed to increase in RTgill-W1 cells in a dose-dependent manner. In contrast, no significant ROS production was detected in OmB brain cells after 24h of exposure. These findings suggest that the tested AdMas can exert mild cytotoxic effects on fish cells, particularly at higher concentrations. However, the absence of ROS production in brain cells and the relatively low levels of toxicity observed suggest that the neurotoxic effects of these materials may be limited under the tested conditions. This work showed that in vitro tests based on fish cells represent a useful tool for ecotoxicological hazard screening of AdMas. These approaches are essential for improving our understanding of the environmental risks associated with emerging materials and for informing regulatory frameworks that ensure the development of safe(r) and more sustainable AdMas. This work was supported by the iCare project (Grant Agreement ID: 1010929071) and the SUNRISE project (Grant Agreement ID: 101137324), both funded by the European Commission.

6.07.P-Th247 Tier 2/3 in vitro methods for early stage-hazard assessment of Safe and Sustainable by Design (SSbD) biocides

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Safe and Sustainable by Design (SSbD) is a design approach for developing and evaluating new chemicals and materials, as well as assessing existing ones, with an emphasis on maintaining functionality while minimizing adverse impacts on human health and the environment. For it, new approach methodologies (NAMs), based on in vitro methods, offer valuable tools for early-stage hazard assessment of SSbD chemicals and materials. In this study, simple (monoculture of enterocytes, Caco-2 cells) and more robust (gastrointestinal epithelial tri-culture model, Caco-2 + HT29-MTX + M Cells) in vitro models were employed to assess the hazards of innovative and conventional biocide alternatives. Both models were exposed for 24 hours to the final product formulations (ranging from 0.03-30%, concentrations used during production and application phases of the products) and to individual compounds (at the corresponding concentrations present in the product formulations), and effects on cell viability (resazurin), inflammatory response (IL-8, sublethal concentrations), and barrier integrity (TEER) and permeability (LY assay) were evaluated. Results showed that both products were significantly cytotoxic starting at 0.03% for Caco-2 cells (innovative was slightly more cytotoxic than reference). In addition, a slight increase of IL-8 release was observed at 0.03% of the innovative product. On the other hand, cytotoxic effects on tri-culture model started at 0.3% (only reference). Inflammatory response showed a significant increase of IL-8 at 0.3% (only reference). Results obtained from barrier integrity of the tri-culture model showed significant decrease in TEER values starting at 0.3% (only reference) and from 3% in both products. In accordance to these results, barrier permeability significantly increased starting at 3% (both products). Overall, results indicated that the toxicity of the innovative product was generally lower than the reference product. In addition, both models showed high sensitivity, however, the tri-culture model was more sensitive and robust than Caco-2 model, indicating that the epithelial tri-culture model could be more suitable for toxicity assessment of the human GIT. This work was supported by the EU Horizon Project “Safe and Sustainable by Design Advanced Materials” (SUNRISE) under Grant Agreement no. 101137324.

6.07.P-Th248 Integrating Predictive Ecotoxicology into the Sustainable Design of Paper-Based Electronic Materials

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The growing demand for environmentally responsible electronics has accelerated the development of paper-based, printed components that offer a sustainable alternative to conventional plastic- and metal-based devices. A key challenge is selecting functional chemicals-such as flame retardants, conductive inks and binders, that provide adequate performance while remaining low in ecotoxicity and compatible with biodegradation. In line with the Safe and Sustainable by Design (SSbD) framework, this study incorporates predictive environmental toxicology into the early formulation stages of new material systems. Initially, we employed a data-driven computational workflow to assess the ecotoxicity of 29 chemical constituents (and their precursors) of the paper based Printed Circuit Boards (PCBs). Experimental data for fish, invertebrates, and algae were compiled, and when data gaps existed, available quantitative structure-activity relationship (QSAR) models and read-across approach were used to generate reliable toxicity predictions. Then, all compounds were classified according to U.S. EPA environmental toxicity categories to enable comparative hazard assessment and support the identification of safer substitutes. Most substances evaluated had sufficient experimental or predicted data to support classification. The resulting hazard profiles ranged from practically non-toxic to moderately or highly toxic, highlighting a subset of chemicals of potential environmental concern. Finally, we assessed the environmental impact of the final product, which is mainly consisted of polymers. It has been demonstrated that components in polymerised form should not exhibit toxicity. Consequently, it is important to evaluate the toxicity of monomers and their precursors because, if polymerisation or recycling is insufficient, they may be released into the environment. Overall, the study underscores the potential of in silico tools to guide the selection of low-toxicity, recyclable components for sustainable electronic materials. Remaining challenges include limited high-quality ecotoxicity data, restricted model applicability for novel chemicals, and the difficulty of evaluating polymers-barriers that must be addressed to fully integrate computational methods into sustainable materials development and regulatory practices.

6.07.P-Th249 Occupational and consumer exposure to VOCs during the production and use phase of renewable Wood Plastic Composites for car interior trims

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Major vehicle manufacturers have adopted the use of bioplastics and bio-based plastics, including natural-fiber composites and engineering plastics fortified with natural fibers, but their application is still limited. Until now, car interior trims are usually made of virgin plastics like Polypropylene (PP) filled with mineral powder like talc, and when introducing new materials, the development is recommended to be done according to the SSbD Framework. The SSbD4Chem project aims to develop Renewable Wood Plastic Composites (WPCs) for car interior trims with the target to reduce Total Volatile Organic Compounds (TVOCs) emissions compared to PP interior trims and assure no risk to human health. WPC based on renewable/recycled PP reinforced with cellulose and wood-based fillers are investigated, focusing on VOC emissions and potential occupational and consumer exposure (Step 2 and 3 of SSbD Framework) ensuring safe use from the early R&D phase. To estimate human exposure to TVOCs from interior trims, testing was done to determine emission factors from the various WPCs (standardized method VDA 278). These WPC included soft wood fibers, fibrous cellulose powder and hemp with content ranging between 15% and 30%. Emission factors from these WPC were compared to commercial PP formulation containing talc (benchmark). ECETOC-TRA Consumer model was parameterized for a car-scenario and interior air concentrations were estimated in different scenarios using the emission factors. Air change rates were varied based on literature data simulating parked/driving, open/closed window and ventilation/no ventilation scenarios. Emission factors from pristine WPC and weathered WPC (UV, humidity and temperature changes) were also used as input to the model. The model predicted TVOC interior air concentrations resulting from WPC emissions to be reduced by a factor of 3-6 compared to the benchmark. Increased air change rates (through open windows, turning on fan and/or no circulation) further decreased the air concentrations by a factor of up to 35 when driving with open windows compared to a parked car with closed windows. Weathering the WPC during a period of 10 days resulted in reduced emissions of TVOC which led to reduced air concentrations by a factor of 2-40 (depending on the WPC) compared to predicted air concentrations based on emissions from pristine WPC. Scenarios covering worker exposure during compounding of the WPCs are currently under development within the project.

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6.07.P-Th250 The ProScale Suite

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The ProScale consortium, formed in 2015, identified ambitions to reduce chemical exposures along the life cycle of products beyond regulatory requirements. Several tools are available for assessing human and ecotoxicological ((eco)toxicity) indicators in LCA, but few focus on near-field exposure. Hence, ProScale was developed and launched in 2017 to fill that gap. Recent studies have shown that there is a need for tools that are easy-to-use and cover all impact pathways. Triggered by that need and the inclusion in the Safe and Sustainable by Design (SSbD) framework led to recent advancements of ProScale.

The purpose of this abstract is to introduce the new advancements of ProScale, including the development of several new modules and their coherence.

The recent advancements and development of new modules allow for full (eco)toxicity assessments in LCA, from cradle-to-grave, taking into consideration both near-field and far-field exposure. The foundation of all modules is that hazard- and exposure information is combined to derive ProScale scores for a product, unit process and/or system. The hazard information originates from GHS while the exposure information is based on exposure modelling recommendations by ECHA in several guidance documents for exposure assessments under REACH (EU Regulation No. 1907/2006).

The original module, ProScale-W was mainly developed for assessing near-field exposure of chemicals for Workers. Recently, recommended default exposure settings for products' end-of-life have been added. Also, the hazard factor scoring has now been developed to include the newest hazard categories under CLP (EU Regulation No. 1272/2008). Further, a separate module for the Service Life was developed, ProScale-SL, to ensure coverage of all life cycle stages. Two more modules have been developed where needed to complete the

desired applicability domains, also to: direct exposure to the environment and indirect exposure to humans. These modules are referred to as ProScale-E and ProScale-IE respectively. The ProScale-IE module advances ProScale from being a tool only focusing on near-field exposure to also account for indirect exposures, i.e., exposure of chemicals to humans via food/water/air intake.

In summary, these modules will allow for a coherent toxicity potential assessment for ecotoxicity as well as human toxicity for both direct and indirect exposure routes in chemical footprint contexts, such as LCAs of products along the whole lifecycle.

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6.07.P-Th251 Extending ProScale to Far-Field Human Exposure: The Indirect Exposure (IE) Module *Efstathios Reppas Chrysositsinos, Pernilla Andersson, Amanda Lundberg, Maja Halling, Rosella Telaretti Leggieri, Therese Kärnman, Ioannis Liagkouridis and Tomas Vilhelm Rydberg, Swedish Environmental Research Institute (IVL), Sweden*

ProScale is a screening-level life-cycle tool for chemical impact assessment that operates on regulatory data, ensuring compatibility with risk-assessment practice. While other ProScale modules target direct human exposure and ecotoxicity potentials, indirect (far-field) human exposure remains insufficiently captured in early-stage SSbD assessments. We introduce the ProScale Indirect Exposure (IE) module, designed to quantify systemic human exposure arising from chemical releases into environmental media across the life cycle.

IE adapts the ProScale-E architecture by combining hazard factors (HF), substance-specific mass flows (MF), and a persistence–exposure relevance factor (PFI,r) for key routes: inhalation via air, ingestion via drinking water, and dietary exposure through food. MF is calculated by coupling product composition with environmental release fractions derived from REACH/ERC scenarios. PFI,r integrates a persistence multiplier (Pdeg), based on standard REACH biodegradation tiers, and a route-specific environmental relevance multiplier (EI,r), determined by physico-chemical triggers for volatility, mobility, solubility, sorption, and bioaccumulation. The combined ProScale-IE score thus maps intrinsic hazard onto conservative, regulatory-consistent estimates of far-field exposure potential.

We apply IE to a retrospective polycarbonate-bottle case study previously evaluated with other ProScale modules, focusing on additives and detergent residues released during use and end-of-life. Environmental release fractions, persistence categories, and EI,r tiers were assigned using only SDS/REACH-level data to mirror realistic SSbD data availability. Preliminary results show that a small number of substances dominate far-field impacts due to high persistence and mobility; while others are associated with volatility-driven inhalation.

We view the IE module as a screening-level chemical-footprinting extension of ProScale with high SSbD relevance. ProScale-IE complements existing ProScale modules, forming a unified screening framework for life-cycle chemical assessment.

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6.07.P-Th252 Does Chain Length Matter? Influence of Molecular Properties on the Ecotoxicity of Alkyl Phosphate Flame Retardants

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Flame retardants are chemicals used in consumer and industrial products to reduce the flammability of e.g. plastics, paints, textiles and fuel. They may, however, also cause cancer, behavioural changes and allergic reactions in test organisms. Alternatives have been designed, but it is yet unclear if these are less toxic to marine biota than the original flame retardants. It has been shown that compounds with shorter carbon chain lengths tend to be less toxic than their longer analogues, but it remains unknown if this also applies to alkyl phosphate FRs. Since this knowledge may aid the design of safer alternatives, the present study aimed to investigate the relationship between molecular properties of alkyl phosphate flame retardants and their ecotoxicity. To this end, the acute (48h) toxicity of five alkyl phosphate FRs to *Artemia salina* nauplii (<24h) was determined, following OECD guideline 202 with slight modifications. The selected compounds included Trimethylphosphate (TMP) Triethylphosphate (TEP) Tripropylphosphate (TPP) Tributylphosphate (TBP) and Tris(2-chloroethyl)phosphate (TCEP). The tests were performed in 24-well plates with 6 concentrations per compound in artificial seawater, including a negative control, and with four replicates per treatment. Log-logistic concentration-effect models were fitted to the 48h mobility data to derive effect concentrations. In decreasing order, the EC50 values were 22416 ug/L for TMP, 8067 ug/L for TEP, 489 ug/L for TPP, 409 TCEP, and 45 ug/L for TBP. All EC50 values differed significantly except for TMP and TEP, and for TPP and TCEP. These results showed that the longer the carbon chain, the lower the EC50 value, hence the more toxic the compound. Yet, TCEP was more toxic than TEP, although they have the same carbon chain length. This can be explained by the chlorine atoms in TEP, which may have increased its ecotoxicity.

TMP was the least toxic compound, thus the most promising alternative, but the flame-retardant efficiency should be considered before application. It is concluded that flame retardants that are chlorine-free and have shorter carbon chains are less toxic to *A. salina*. These findings can facilitate the design of safer alternative flame retardants.

This poster was developed by students as the final product of a BSc-level course on Ecotoxicology, and won the best poster prize in the assessment of the course.

6.07.P-Th254 Implementing Safe and Sustainable-by-Design Strategies in the Development of TiO₂@Surfactin for Cosmetic Application

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Advanced Materials (AdMa), including engineered nanomaterials, offer novel functionalities but require early-stage risk assessment if an SSbD approach is followed, to ensure their safe and sustainable development. The European Commission's Safe and Sustainable by Design (SSbD) framework supports this goal by integrating hazard, occupational exposure, and application-related exposure considerations from the design phase onward. Within the SUNRISE project, representative AdMa were selected to demonstrate the implementation of the safety part of SSbD. The case study focused on three TiO₂-based AdMa functionalized with surfactin (P25@SS, TiO₂@SS-1, and TiO₂@SS-2), developed as UV filters for sunscreen formulations. A qualitative questionnaire was first administered to the company in order to identify safety-relevant data gaps, which guided subsequent physicochemical characterization using techniques such as TEM, ATR-FTIR, TGA-DSC, BET, DLS, ELS and CSA. The characterization highlighted relevant differences among the AdMa. P25@SS showed larger particle size and lower surfactin coverage compared to the TiO₂@SS-1, and TiO₂@SS-2 alternatives. Despite similar coating percentages for TiO₂@SS-1 and TiO₂@SS-2, DSC data suggested different SS arrangements, consistent with BET results showing a higher surface area for TiO₂@SS-1. FTIR confirmed effective surfactin coating across all samples.

After optimizing stock dispersions in ultrapure water, the colloidal behaviour of the AdMa was evaluated in media relevant for human (sweat, saliva) and environmental (freshwater, marine water) exposure, as well as in cell-culture media supporting (eco)toxicological testing. All SS-functionalized materials showed stable dispersions and negative surface charge, with zeta potential values decreasing in complex media but without marked effects on sedimentation behaviour, indicating the stabilizing role of the coating. These data supported the interpretation of the preliminary ecotoxicity tests on zebrafish embryo assays and algae growth inhibition, which showed no acute toxicity in freshwater organisms.

Overall, the case study demonstrates how a structured, questionnaire-driven workflow can guide targeted testing and support the early application of the SSbD framework to AdMa design.

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6.07.P-Th255 Implementing the Joint Research Centre's (JRC) Safe and Sustainable by Design (SSbD) Framework to Guide the Development of Novel Bio-Based Flame Retardants Within the BIOSAFIRE Project.

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Flame retardants play a key role in reducing fire damage. However, despite their intended benefits, growing evidence shows that many of these substances pose significant risks to human health and the environment. Numerous flame retardants are known to be problematic due to their persistence, bioaccumulation, and adverse effects such as neurotoxicity and endocrine disruption. Their widespread detection in air, dust, drinking water, food and recycled materials further underscores the need for urgent action.

Given their structural features and potential as fire-resistant materials, lignins and tannins have attracted rising attention. Building on this, the EU-funded BIOSAFIRE project aims to create new high-performance bio-based flame retardants capable of replacing current hazardous options. A central element of the project is the continuous integration of the Safe and Sustainable by Design (SSbD) approach throughout the innovation process, from material selection to end-of-life considerations. Applying the SSbD framework will clarify how early hazard screening, life-cycle perspectives and sustainability requirements guide design and development decisions.

To demonstrate improved safety and sustainability, project outcomes will be benchmarked against existing alternatives. Five case studies across four major industrial sectors —naval, rail, household appliances and construction— will later be used to validate and refine the framework. At this initial stage, work has concentrated on identifying and characterizing candidate materials while preparing appropriate testing strategies in accordance with SSbD principles. Efforts have been focused on the definition of the intended function, preliminary safety and sustainability assessments, and the planning of early design and testing. Some preliminary safety-related assays have already begun in parallel, although still at an exploratory level. This structured approach ensures that considerations of safety, performance and sustainability are embedded from the very beginning.

BIOSAFIRE is thus poised to advance safer fire-protection solutions in Europe, supporting not only the development of innovative flame retardants but also the broader adoption of an innovation framework applicable to other chemical sectors

6.07.P-Th256 Innovative Furniture Design Solutions for Eliminating Chemical Flame Retardants

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The UK's current prescriptive furniture fire safety requirements contribute to the UK's public, wildlife and environment having some of the highest flame retardant levels in the world. The commonly used brominated flame retardants and organophosphate flame retardants are of major concern and under careful scrutiny by the European Chemicals Agency. The legal requirement for flaming ignition source testing is met by the use of chemical flame retardants in upholstered furniture. This exposes people and the environment to potentially harmful chemicals and hinders product circularity, without any clear evidence of a fire safety benefit.

Innovative design solutions offer opportunities to reduce or ideally eliminate the use of harmful chemical flame retardants in furniture products but the UK's furniture fire safety regulations do not account for modern furniture construction methods, such as the use of fire barriers materials. Other solutions include the use of safer alternatives to toxic chemicals. For example, use of naturally flame resistant wool or treatment of upholstered furniture fabrics with low toxicity expanded graphite instead of persistent brominated flame retardant chemicals to achieve product flame retardancy. In addition to effective policy changes and intelligent design solutions,

Fidra believe that we can reduce or eliminate the use of harmful chemicals through enforced product chemical labelling to disclose chemical flame retardants present in materials throughout supply chains and through implementing extended producer responsibility schemes.

In summary, this presentation will outline the growing research evidencing the environmental impacts of chemical flame retardants used in furniture products, the current regulatory barriers to reducing flame retardant use in the UK, sustainable design solutions being developed by progressive industries as well as the challenges associated with managing furniture waste containing persistent chemical flame retardants. Findings presented will help inform policy, research and furniture supply chain industry innovation developments needed in the future to support sustainable by design solutions and public and environmental protection from harmful chemicals.

6.07.P-Th257 Implementing Safe-and-Sustainable-by-Design for Perovskite-Silicon Tandem Solar Cells
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The SSbD (safe and sustainable by design) framework recommended by the European Commission aims to minimize risks of safety and sustainability in new materials by a multi-perspective approach. To date its practical application in solar cell development is emerging but still in early stages with many open questions regarding precise assessment strategies or how to consider all steps along the life cycle. Perovskite solar cells have great potential for versatile applications and due to their high-power conversion efficiency; however, most perovskites are lead-based materials posing substantial safety and sustainability concerns. By applying a tailored scoping strategy aligned with the revised SSbD framework we aim to identify and reveal the environmental and safety hotspots along all process steps of the tandem solar cell module. Thereby the study will deliver a solar cell with reduced lead-content, more sustainable chemicals and reduced risk for workers and users without trade-offs in the performance. Regarding the safety and environmental assessment, the full production processes will be scanned regarding potential risks in the relevant categories, predefined in the scoping. Preliminary analyses identified lead-content and oxidizing solvents as hotspot in the material use, so alternatives will be identified and evaluated. Regarding process safety a detailed investigation will check the safety concerns and risks of all required steps, e.g. the spraying process and the plasma crystallization step. Mitigation strategies for identified risks will be designed and implemented in close collaboration with the technology developers to ensure fast and lasting effectiveness. In addition, the potential to reduce environmental impact of the final product will be attempted by considering measures to maximize resource efficiency for different fabrication techniques, and designing the solar cell module with best fit for recycling and circularity. By uniting the different assessment dimensions of SSbD we help to guarantee environmental compatibility and safety for the whole production process, as well as to include considerations regarding the end-of-life and the recyclability right from the start. The applied scoping and assessment strategy will be adaptable to similar technology and material development processes and help to advance the field of solar cell technology, the implementation of SSbD and associated strategies for safety and sustainability assessments.

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6.07.P-Th258 Towards Ideal Safe and Sustainable Home Batteries: Criteria and Application
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Societal background

Batteries are key to achieve the Green transition. Last year in particular the development and use of home batteries is booming within the field of the energy transition. The ideal home battery is safe, sustainable and circular, without the use of hazardous or critical materials. However, a clear description of parameters that constitute the ideal battery is lacking, despite many developments in battery technology. This hampers policy makers as well as innovators in decision-making, as it lacks focus and clarity on weighing pros and cons of decisions.

Envisioned result and impact

In general, regulators formulate their criticisms well, but describing products in terms of what would be ideal seems to be more challenging. In this project we apply an innovative approach to translate parameters set by an ideal product into a set of concrete criteria. We have tested this approach on home batteries. These types of batteries are currently heavily promoted, but are still surrounded by high uncertainties about their composition, their risks, safe use and environmental impact. On one hand we defined properties for an ideal battery in terms of functionality, safety, sustainability, circularity (including critical materials) and cybersecurity, based on criteria in Safe-and-Sustainable-by-Design approaches like the JRC SSbD framework (2025). These criteria are specified in more detail based on literature and important characteristics of home batteries. On the other hand battery types were selected that are currently used and are expected in the near future. This was done based on input by suppliers, producers, knowledge organisations and literature. Five type of batteries were selected: lithium-iron phosphate (LFP), lead-gel, Nickel Manganese Cobalt (NMC), salt and solid-state batteries. The definition of an ideal home battery in combination with an assessment of the selected type of batteries, will give direction for further efficient and effective home battery technology development. This approach might form a basis for application for other energy transition technologies, for SSbD for batteries, and to develop knowledge to support current safety and sustainability regulatory frameworks. The result of the project is a draft practical approach including criteria how to address functionality aspects, safety, sustainability, circularity and cybersecurity and a comparison of the selected home batteries based on these parameters.

6.07.P-Th259 From Lignin to BPA-Free Epoxy Resins: Implementing Safe and Sustainable by Design in Polymer Innovation

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Bisphenol A is widely used in epoxy resin production, yet its endocrine-disrupting properties and potential human and environmental contact throughout product life have prompted the search for safer, sustainable alternatives. This need is particularly relevant in construction and infrastructure applications, where coatings undergo prolonged mechanical and environmental stress that may lead to material degradation and release of chemical constituents. The LEGACY project addresses this challenge by developing fully biobased, bisphenol A-free epoxy systems derived from lignin and evaluating their safety within the integrated Safe and Sustainable by Design (SSbD) framework.

The project aims to determine whether lignin-based epoxy intermediates can replace bisphenol A while reducing intrinsic hazards and limiting exposure across the material life cycle. Early-stage material development is integrated with systematic safety evaluation. Hazard assessment combines physicochemical characterization, computational modelling, read-across analyses, and non-animal in vitro assays targeting key human health and ecological endpoints, including endocrine activity, irritation, skin sensitization, developmental effects, and aquatic toxicity. Exposure assessment examines potential release and contact during coating application, occupational handling, use-phase wear, and end-of-life scenarios through scenario analysis, controlled emission studies, and environmental distribution modelling.

Sustainability aspects are considered to ensure hazard and exposure reduction aligns with broader environmental objectives. This integrated approach supports the identification of promising biobased structures and formulation strategies that achieve functional performance while minimising risk.

By integrating hazard, exposure, and life cycle considerations into early material design, the project contributes to advancing SSbD practices in polymer development. This work offers a pathway to reduce reliance on bisphenol A in epoxy resin applications and provides a framework for evaluating emerging biobased materials in a manner that supports regulatory alignment, industrial feasibility, and long-term environmental protection.

6.07.P-Th260 Implementing the SSbD framework to support the development of bio-based sustainable surfactants

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SurfToGreen is an innovation action aiming to develop a new portfolio of fully bio-based surfactants for key applications. By using renewable biomass-derived building blocks sourced from EU agricultural and forest side

streams, the project seeks to create surfactants with up to 95% bio-based content, overcoming the current performance and production throughput constraints. Thus, SurfToGreen seeks to provide environmentally friendly alternatives to conventional, petroleum-based products, thereby contributing to a more sustainable future.

Safety and sustainability of innovative solutions are assessed following the EC Safe and Sustainable by Design (SSbD) framework. More specifically, hazard assessment, occupational, environmental and public health risk assessments, and Life Cycle Assessment are performed throughout the entire value chain of SurfToGreen biosurfactants in specific applications, through the creation of specific thresholds and criteria. Techno-economic and social acceptance assessments are also foreseen, to support the decision making process in SSbD. The assessment will be validated engaging partners and their networks covering all the surfactant value chain, including feedstock producers, chemical manufacturers, and end-users (large, medium and small enterprises). The project will validate the effectiveness, sustainability and safety of its bio-based formulations in industrial demonstrators for home and personal care, textile enhancement, and agriculture.

Disclaimer/Disclosure:

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6.07.P-Th261 From Control Banding to Integrated Assessment: A Dual Risk and Environmental Approach Applied to Proton Exchange Membrane Electrode Fabrication

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The move toward Safe and Sustainable by Design (SSbD) requires methodological frameworks that can address safety, environmental performance and functionality at early stages of material and process innovation. In this work, we focus on the application of a control-banding-based Chemical Risk Assessment (CRA) as a practical tool to evaluate safety aspects during development and scale-up, and on its complementarity with Life Cycle Assessment (LCA). Rather than treating LCA and CRA as isolated assessments, the study explores how both types of indicators can be jointly mobilized to support SSbD decision-making.

The approach is tested through two electrochemical manufacturing case studies: porous electrode fabrication for Proton Exchange Membrane systems and catalyst-coated electrodes for Proton Exchange Membrane Fuel Cells, including end-of-life recycling. These systems traditionally rely on platinum-group metals; the substitution of Pt with Ni-based or emerging catalyst formulations is assessed through a dual lens: (i) worker and process safety through control banding, and (ii) potential environmental impacts through LCA.

The control banding methodology is applied at successive development stages, enabling rapid identification of chemical hazards, exposure determinants and mitigation priorities, even when full toxicological or exposure datasets are not available. LCA is performed in parallel to capture environmental trade-offs across the life cycle, and the combined outputs are positioned within the Joint Research Centre's SSbD framework to map points of convergence and divergence between safety-oriented and environment-oriented criteria.

Results highlight that integrating CRA control banding and LCA from the earliest phases of innovation supports more robust substitution decisions, avoids late-stage lock-ins, and contributes to a more concrete operationalization of SSbD in industrial settings. The work demonstrates how control banding can act as an entry point for safety assessment within SSbD, while maintaining methodological complementarity with LCA.

Disclaimer/Disclosure:

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6.07.P-Th262 Data quality, data gaps and functionality: Development of a transparent safety assessment framework within the BIORING project

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The EU project BIORING 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.

Implementing Safe and Sustainable by Design (SSbD) in the BIORING project involves key challenges, particularly in meeting data and criteria requirements during early innovation phases. A major limitation is the lack of data on biomass conversion processes into bio-based chemicals. Here, we develop and apply a strategy to include data quality and data gaps in the chemicals hazard evaluation to support SSbD integration and support decision-making for more safe and sustainable chemistries in a transparent manner.

The safety assessment focuses on components used in the production of biobased products, and includes catalysts, by-products, and other process-related substances. The assessment draws on existing knowledge such as review papers, databases, and regulatory sources, and uses in silico tools to address data gaps as far as possible. The goal is to support project partners in identifying and transitioning to safer alternatives, in line with the SSbD Framework. Experimental and in silico data are integrated into a harmonized safety score, while remaining data gaps are clearly communicated to guide further decision-making with regards to selecting appropriate management options. Final decision-making also considers the intended functionality and use context of each chemical or material, alongside broader criteria such as Green Chemistry principles.

Disclaimer/Disclosure:

BIORING is a Circular Bio-based Europe Joint Undertaking funded project (2023-2026, GA 101112379)

6.07.P-Th263 Data Requirements for the Redesign of Mineral Carbonation Process with the ‘Safe and Sustainable-by-Design’ Principles

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In 2024, the European Commission Joint Research Centre introduced methodological guidance for implementing Safe and Sustainable-by-Design (SSbD) principles, aiming to integrate safety, environmental, economic, and societal considerations into the development of chemicals, materials, and processes. This framework represents an evolution of Life Cycle Sustainability Assessment (LCSA) by explicitly incorporating safety dimensions. However, practical applications remain scarce, and case studies demonstrating SSbD integration are limited. This study addresses this gap by identifying essential data requirements for conducting Life Cycle Assessment (LCA) aligned with SSbD principles and proposes a process-flow approach to integrate environmental impact calculations with SSbD assessments. The methodology is applied to a case study on mineral carbonation of steel slags as a carbon capture, utilization, and storage (CCUS) technology. Beyond assessment, the study explores redesign strategies for the current mineral carbonation process to enhance alignment with SSbD principles, focusing on reducing energy intensity, optimizing material flows, and minimizing occupational hazards. The outcomes provide a practical roadmap for generating and structuring data to meet SSbD criteria while informing process improvements for safer and more sustainable CCUS technologies. Future work will extend to economic and social dimensions for comprehensive sustainability evaluation and apply the proposed approach in real-world projects to advance safe and sustainable manufacturing value chains.

6.07.P-Th264 SSbD4SME: A Digital Tool Assisting SMEs in the Development of Safe and Sustainable Products

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Developing or re-designing products according to the Safe and Sustainable by Design (SSbD) Framework requires multi-disciplinary expertise. Small and Medium Enterprises (SMEs) often lack in-house expertise in one or several disciplines (i.e., hazard, risk and life cycle assessments) needed to perform a full SSbD assessment. Therefore, the aim of the SSbD4SME project is to develop a digital screening tool that will assist

SMEs in selecting suitable alternative chemicals to replace problematic ones (reference) in existing products or to ensure safe and sustainable chemicals are used when developing new products.

The SSbD4SME tool contains several modules covering the first 4 steps of the SSbD Framework: 1) hazard characterization, 2&3) human and environmental exposure and risk assessment during the production and use phase and 4) life cycle assessment (LCA). In order to make the SSbD4SME tool user-friendly for SMEs, information from existing databases will be used when available to minimize data input by SMEs and default scenarios allow for easy use of the tool. The current version of the tool contains pre-set human and environmental exposure scenarios for chemicals used in textiles and LCA scenarios for textile products. By entering a chemical CAS number, hazard information on the existing (reference) and alternative chemicals will be collected from online databases and the resulting SSbD-score will be calculated by the tool. An exposure and risk assessment is performed using CHESAR with the required chemical input obtained from existing database (obtained again via CAS number), and pre-set exposure scenarios for chemicals used for finishings and coatings in textiles. SSbD-scores are generated for reference and alternative chemicals based on the risk characterization ratios (RCR). The LCA module focuses on textile products and can be applied for a 'cradle to gate' (impact of the manufactured textile product) or 'cradle to grave' (impact of the manufactured textile product including its use phase and disposal) LCA, depending on the product development stage. SSbD-scores will be generated for different environmental impact categories. The SSbD4SME tool also contains a database of alternative chemicals that can assist SMEs in selecting chemicals to substitute problematic ones. The SSbD4SME project will provide SMEs with a screening tool that can assist them during the (re)development stage of (new) products in selecting safe and sustainable chemicals.

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6.07.P-Th265 Bridging Science and Industrial Innovation: Implementation of the Safe and Sustainable by Design (SSbD) Framework in the Development of Alternatives to Substances of Concern in the DESIDERATA project

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The operationalisation and testing of the Safe and Sustainable by Design (SSbD) framework is taking place in full scale since the adoption of the Recommendation by the European Commission in 2022. The DESIDERATA project inherently incorporates the SSbD framework as the core of the industrial innovations for the development of alternatives to Substances of Concern (SoC) with plasticising, flame retardant and surfactant properties.

We demonstrate how the SSbD framework is implemented and operationalised in five Use Cases for the development of alternatives in the lubricants, polymers, electronics, additive manufacturing and construction sectors. Targeted Substances of Concern include medium-chain chlorinated paraffins, halogenated flame retardants, antimony trioxide, polytetrafluoroethylene (PTFE) and aluminium powders. Preliminary results on the safety and sustainability assessment of the SSbD alternatives are discussed, which are based on the application of OECD validated methodologies, New Approach Methodologies (in silico/in vitro), standardised Life-Cycle Assessment methodologies and more. Tiered approaches are employed where possible, to support the application of targeted techniques that allow the identification of hotspots and employment of alternative development routes/corrective measures along the value chain and the lifecycle stages of the DESIDERATA products.

Proof of Concept for the implementation of the SSbD framework is under development, which aims to integrate safety and sustainability (environmental and socio-economic) with functionality and technical performance, without compromising investment and R&D practices in industrial settings. We discuss how critical decision points should be incorporated in the implementation of the framework and how the SSbD practitioners may align the work with technical developers within interdisciplinary environments. Overall, the integrated approach developed in the DESIDERATA project aspires to ensure regulatory alignment, enhance practical applicability, and support systemic transitions towards safer and more sustainable chemicals and materials.

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6.07.P-Th266 Critical evaluation of the SSbD framework to support early stage innovation
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The SSbD framework is conceived to support innovation towards a safer and more sustainable society. While the framework contains guidance on the evaluation of innovations on the basis of hazard, risk and impact, it fails to provide specific handles how to support the innovator in the innovation project.

On the basis of two concrete innovation projects, one TRL1-3 (FONT project) and a follow-on project (RADAR project) at TRL 4-6, the SSbD framework and the proposed updated framework are evaluated. In these projects four innovations are developed in collaboration with industry partners.

Specific tools and approaches were developed to allow interaction with the innovator along the innovation project, tools for data structure over the lifecycle and tools to handle the multi stakeholder aspect of the SSbD support of an innovation project.

In the specific projects innovators, scientists and industry were represented.

Overall, within the two projects, the concept of the SSbD integrator has been developed. This innovative role assures the translation of the SSbD framework in a workable project approach.

6.07.P-Th267 Tailoring, Tiering and Archetypal Scenarios: Outcome of two Workshops on SSbD Scenarios in the Network for Safe and Sustainable Chemicals & Materials (NSC)

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The term “SSbD scenario” was integrated into the revised SSbD Framework, where it serves as bridge between the SSbD scoping and a tailored safety and sustainability assessment. A first NSC workshop (NSC: Network for Safe and Sustainable Chemicals & Materials) in June 2005 explored the description of the SSbD scenario, comprising aspects of the SSbD scoping and aspects of the business case and project plan. The ideal SSbD approach proceeds from simplified to intermediate to full SSbD, if the project passes the respective gates into lab phase, pilot phase, launch phase. But not all projects operate in a context allowing that tiered SSbD approach in full swing. A second NSC workshop in December 2025, using well-established economic metrics of R&D pipelines, explored the tailoring of SSbD approaches to selected case studies, and derived also archetypal scenarios that can serve as template for innovators to adapt their SSbD approaches. Scenarios are tailored based on the novelty (disruptive or incremental), the sector of use, and the objective (or value proposition: safer, more sustainable, cheaper, more performant, system-integrated, etc), the R&D budget and the immediacy (TRL, time to market). One scenario showed the full potential of SSbD: a relatively big project (budget of 1mn€/y) with disruptive technology in a sensitive lifecycle value chain and big market potential. Even here, tiering and prioritization is essential, and then allow several rounds of re-design with both safety and sustainability assessment of increasing maturity. For all other scenarios, with more typical market potential (net present value of e.g. 5mn€) and more modest budget (e.g. 250k€/y), the scope of the SSbD WP has to be strongly prioritized according to the specific SSbD pull, to the novelty, and to specific objectives. The essential requirement to a positive Return on Investment of implementing SSbD work packages is a strong SSbD pull by the downstream lifecycle actors. It can compensate for all other impacts. This goes as far that for innovation cases in cosmetics and pharma, the probability of commercial success is 0% without SSbD, and safety screenings are done by all competitors. The proposed and tested methodology suggests that SSbD practices will first permeate industries in end-consumer markets, and those with a high environmental footprint, because SSbD pull is high there. However, incremental innovations will remain very selective in their SSbD tailoring.

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6.07.P-Th268 A certification Scheme for Safe and Sustainable by Design: Fulfilling the Needs of Industry

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Safe and Sustainable by Design (SSbD) applied to chemicals and materials is becoming increasingly recognised as an essential element underpinning the full value chain from blueprint through sourcing of raw materials to development and testing, to market readiness of a product, while supporting regulatory preparedness for industry. The Joint Research Centre's SSbD framework and guidelines promote a tiered approach considering various innovation options or the Technology Readiness Level (TRL) level of the chemical/material, which is in part mirrored by the Integrated Impact Assessment Framework being developed as a practical support tool in the Horizon Europe HE project SUNRISE. The public consultation results on the implementation of SSbD, available in 2025, show high stakeholder interest, revealing the need for further guidance, better access to tools and an increasingly unified approach to safety and sustainability assessments.

SSbD is not enshrined in any regulation. SSbD implementation does not equate to regulatory compliance, but clearly industry would benefit from a certification scheme demonstrating SSbD adoption. An innovative scheme is under development as part of the SUNRISE project, comprising a first step of self-assessment based on a questionnaire following the structure of the JRC SSbD framework and covering all stages of the product life cycle. Industry must justify whether their solution improves, worsens, or does not change key factors compared to mainstream alternatives e.g., including critical raw material use, human exposure, recyclability, material efficiency, durability, and overall environmental performance. Industry should provide justification (improvement, deterioration or neither of both, in comparison to the main stream alternative) for a range of factors including phase out the use of Carcinogenic, Mutagenic and Reprotoxic (CMR) substances, reduce human exposure as well as enhance recycling, material efficiency, durability and environmental performance. Evaluation and certification will be carried out by an independent and recognised authority, enabling the industry to claim SSbD compliance for a specific product.

Disclaimer/Disclosure:

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6.07.P-Th269 A Matrix-based Scoring System for Selecting Safe and Sustainable by Design Solutions in a Bio-based Coatings Development

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There are very few polymer solutions available on the market that combine energy effective UV curing manufacturing, high biogenic content, and performances in line with demanding industrial sectors, such as automotive, furniture and construction.

The BIORING project addresses this challenge by using the safe and sustainable by design JRC framework to rethink the chemistry of UV curable polyurethane systems and develop a novel portfolio of monomers, oligomers, and biobased coatings.

We apply an iterative approach to data gathering, increasing the level of knowledge, and addressing data gaps, along the development phases of the chemical materials.

The final goal is to compare several alternatives to identify the most SSbD-compliant solutions. We develop a matrix-based scoring system that integrates multiple dimensions, including safety, sustainability, circularity, performance and economic aspects. Each dimension is represented by a set of indicators selected from SSbD strategies, such as hazard assessment (e.g., harmful substance criteria), occupational safety (e.g., exposure levels), consumer safety (e.g., chemical release), life cycle analysis (e.g., climate impact, land use, resource efficiency), life cycle costing, and measures of circularity (e.g., CTI, recyclability).

For each BIORING alternative, indicators are assigned a score (s?) and a weight (w?). The overall SSbD score is calculated as $SSbD\ Score = (w \times s)$ and then converted into a readiness label, enabling comparison across alternatives.

The matrix is applied to the BIORING demonstrator, a biobased coating for automotive interiors, using the data (both quantitative and qualitative) generated from the BIORING SSbD assessment, allowing alternatives to be compared through a consolidated score. As BIORING is a low-TRL project (TRL 3–5), there is still substantial room for improvement and further development of our solutions. For this reason, intermediate scores are kept visible within the system to pinpoint hotspots and provide actionable guidance for improving overall performance.

One key outcome of the BIORING process will be a White Paper giving learning and recommendations to apply

the SSbD framework to biobased coatings

The Bioring is a Circular Bio-based Europe Joint Undertaking funded project (2023-2026, GA 101112379).

6.09.P Risk Perceptions, Risk Communication, and Public Trust in Environmental and Risk Assessment

6.09.P-Th270 Parameter Influence on Environmental Impact Targets in Battery Production: A Systematic Sensitivity Approach

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Reducing the environmental impacts of products like electric vehicle batteries to stay within humanity's safe operating space necessitates target-based approaches and an understanding of parameter influences on target variability. This study conducts a systematic sensitivity analysis to identify key parameters influencing climate change impact targets for battery production, using Sobol first-order indices to decompose target variability. The parameters considered include national climate change impact budgets, allocation factors at various levels (sector: mobility and product: battery), vehicle lifetime (modeled via a Weibull distribution with varying shape and scale parameters), battery capacity, and number of vehicles. The analysis spans temporal (2030, 2035, 2050), spatial (Germany, Canada), and technical (34–122 kWh capacities) scenarios. Considering the parameter ranges and distributions, the results show that vehicle lifetime is the dominant driver of variability, followed by national budgets and mobility sector allocation factors. These insights enable battery producers in strategic planning by focusing on parameters affecting environmental impact targets such as lifetime but also focus on taking action to harmonising industry wide applicable budgets and allocation factors.

6.09.P-Th271 What Level of Protection Does the Public Prefer in Setting Environmental Water Quality Benchmarks for Aquatic Life? A Nationwide Web Survey in Japan

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Understanding what levels of biological protection the public prefers is essential for effective environmental management, for example when setting water quality benchmarks for aquatic life. However, it remains unclear which biological levels of organization people believe should be protected. To address this gap, we conducted a nationwide online survey in Japan (n = 1211) and asked participants to choose their preferred level of biological protection, ranging from sub-individual responses such as DNA or cellular damage to higher-level effects such as the extinction of multiple species. Surprisingly, more than half of the respondents selected the sub-individual level of protection, suggesting a preference for a stricter protection level than those commonly accepted in actual management (i.e., the population level or higher). The results also suggest that individuals with stronger conservation attitudes, a greater perceived risk of chemicals, a stronger connection to nature, a greater ability to identify species, and older age were more likely to prefer stricter protection. Despite the possibility that the preference for stricter protection stems from misunderstandings of sub-individual level effects, our findings suggest that regulators and environmental managers should recognize and address the gap between public preferences for biological protection levels and the protection goals commonly adopted in environmental management, particularly in stakeholder engagement settings.

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6.09.P-Th272 A Review of Default Assumptions Used in Bird and Mammal Risk Assessments for Seed Treatments and Potential Mitigation Options for the UK

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Under retained EU legislation, the UK Health and Safety Executive's Chemicals Regulation Division (HSE CRD) requires assessment of potential impacts from pesticide active substances and plant protection products (PPPs) on wild birds and mammals.

Pesticide seed treatments offer a targeted crop protection approach with multiple advantages: reduced reliance on foliar applications, protection during vulnerable early growth stages, and potentially lower overall environmental exposure. However, these products frequently fail authorization due to unacceptable acute and chronic risks identified during risk assessment (RA). This stems from conservative default assumptions - particularly that availability of treated seeds on the soil surface is high and that treated seeds and emerging seedlings contain high pesticide concentrations while representing attractive food sources for wildlife. Consequently, an increasing number of PPPs are being withdrawn during re-registration processes, raising concerns about UK food production resilience. At the same time there is an increasing public awareness of chemical exposure as a risk to wildlife.

This HSE CRD project investigated the potential for evidence-based refinements to the RA, which could maintain robust environmental protection whilst maintaining availability of safe and effective products supporting UK crop production and yield.

The project examined the following data sources: publicly available research routinely cited in seed treatment RAs; contemporary research obtained from the updated EFSA bird and mammal guidance (2023) and provided by key stakeholders; and peer-reviewed publications identified through data mining. All relevant citations underwent reliability and relevance assessment, followed by focused discussions on critical aspects of seed treatment RA methodology.

The project report will be publicly released, with ongoing discussions planned regarding future data generation needs. These efforts aim to enhance transparency and stakeholder confidence in the UK RA framework, ensuring decisions are grounded in current scientific evidence while balancing agricultural productivity requirements with environmental protection imperatives.

6.09.P-Th273 Pesticide Fates and Effects on Environments in the Northern Zone

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Plant protection products (PPPs) are used to protect crops from pests in agriculture, forestry and horticulture. Concerns regarding the environmental impacts of pesticide residues occurs. We want to address these concerns, considering the unique climatic conditions in the Northern zone of European Union (EU) which together with agricultural practices used influence pesticide behaviour and persistence, but also risks and risk mitigation methods for approved PPPs. Information exchange between authorities within the Northern European zone is important because of similar climatic, agricultural conditions and practices, which are different from those in Central and Southern parts of Europe. As the data on use, residues and risks of pesticides are currently dispersed across different databases of various authorities and researchers, and are published mostly in national publications, there is a great need to collect, analyze and publish this information as one compact resource.

The aim of this project is to assemble and analyze existing data on pesticide use, their residues and risks of pesticides, but also risk mitigation strategies for approved PPPs, in the Nordic and Baltic countries. Using this consolidated information, we will evaluate potential strategies to reduce pesticide risks to the environment. We identify data gaps and future research steps needs and make recommendations on how to reduce pesticide related risks. The outputs of this project will close knowledge gaps, enhance the development of risk assessment processes and reduce the risks of pesticides in the Nordic and Baltic countries and in the EU.

Results will be disseminated in a scientific publication, which will be communicated to scientific audiences and to the public in constituent countries in 2026-2027.

Disclaimer/Disclosure:

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6.09.P-Th274 Enabling regulatory validation and innovation: Precaution, risk, “Toxic Ignorance Cycles”, and New Approach Methodologies

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Regulators in chemicals management across jurisdictions face significant challenges as they move away from vertebrate animal testing and realise the transformational potential of New Approach Methodologies (NAMs). While NAMs offer a promising new means of (eco)toxicological knowledge production for decision-makers, uncertainties remain, slowing adoption. However, regulatory scientists have long faced an uphill battle against uncertainty in decision-making contexts, and it is noted that in some regulatory contexts what is referred to as uncertainty can hide the distinction between uncertainty, risk, and ignorance. In this presentation we consider innovation in regulatory science broadly and offer some overarching observations from social science and policy perspectives that relate to the impact of risk perceptions in regulatory environments. We provide high-level insights from three related mixed methodology studies (involving interviews, policy analysis, and participant observation) that offer insight into NAM validation processes and implementation barriers for actors across regulatory, academic, and industry environments. Specifically, we report on: 1) how uncertainty and ignorance impact regulatory (non)decisions and (in)action in risk assessment; 2) how certain types of precautionary mindsets may present institutional barriers to NAM acceptance and adoption in risk assessment; and 3) how actors involved in NAM validation act as Institutional Entrepreneurs to navigate change. Together, the observations and recommendations from these mixed method studies utilise the NAMs case to offer insight into how risk perceptions impact regulatory innovation more broadly. We illustrate how greater consideration of risk perceptions, risk complexity, interpretations of risk and precaution, and an awareness of toxic ignorance cycles, can enable stronger alignment between regulatory objectives and policy implementation.

6.10 Translating Complexity – The Role Science Communication Plays in Generating Lasting Societal Impact

6.10.T-01 Clear, Concise, and Complete: Finding the Sweet Spot for Detail and Simplification in Science Communication

Katharina Lang-Hogrefe, Beatriz Alfaro Serrano, Evgenya Zeevi, Matiss Reinfelds, Johanna K. Scheper and Andreas Falk, BioNanoNet Forschungsgesellschaft mbH, Austria

Scientific communication is an important tool to ensure that innovation hits the ground and reaches the relevant audience. Next to scientists this also includes regulators, policy makers, but also the broad community, especially for topics with wide outreach like safety and sustainability. For advanced materials their complexity requires clear and specialised yet accurate communication that balances necessary simplification with the preservation of essential scientific information. Often the communicated outcomes of research are not clear for “outsiders”, do not deliver actionable information and therefore miss trust and impact in the population. This study will compare different communication tools and methods (videos, interviews, visual aids, articles etc.) and analyse their success for various media and communication strategies. A special emphasis will be placed on ‘infographics’, static visuals providing ‘easy-to-grasp’ information for a broad audience. We will illustrate the working principles of different classes of chemicals like flame retardants and surfactants. In this format different familiar symbols (flames, helmets, emojis etc.) are used to illustrate aspects like danger and safety and allow for an intuitive and quick understanding of complex issues. Preliminary results show that infographics are an effective tool to communicate matters of safety of chemicals and materials. The impact of infographics on LinkedIn will be compared with other shared content like video materials and text-based postings. Additionally, also an investigation is attempted on whether individuals reacting to posts including infographics are directly involved in the projects, affiliated with partner institutions, or originate from external audiences, to quantify the outreach of the content and the diversity of the audience as a first indicator for social acceptance. Further on, the use of infographics for other purposes like websites or a printed materials will be evaluated. In addition, we will share lessons learned from several research projects in the field of chemicals and advanced materials. To conclude, breaking-down complexity requires the minds of different stakeholders to extract the essential information. Simplification needs to be handled with delicacy, because generic content will not gather attention or interest. Finally, every means of communication needs to be tailored to the respective audience and balanced with regard to length, detail and precision.

Disclaimer/Disclosure:

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6.10.T-02 Science communication to the general public through younger generations

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Scientific thinking fundamentally differs from general public perception, particularly in considerations of probability and interdisciplinarity. It is essential to understand the complete chain of events: from the sources and concentration of a substance in an environmental matrix, through exposure routes, to its potential harmful effect on health. How can we effectively communicate this complex, data-driven, and probabilistic perspective to a general audience?

One possibility is to educate young people, specifically high school students, and give them an opportunity to experience being a scientist. To accomplish this, we created a highly practical summer school to transform students into active scientists, allowing them to gain a deep, experiential understanding of the complexity of scientific work and its societal relevance.

This work describes an innovative outreach initiative, the "Environment and Health Summer School" (EHSS), organised by RECETOX at Masaryk University, Czech Republic, in cooperation with the science learning center Bioskop, Masaryk University, Czech Republic. The primary objective is to engage high school students (age 16-20) by providing a hands-on, authentic scientific research experience. The programme simulates the full scientific method, from learning fundamental information through conducting a small case study (defining hypotheses, sampling, lab work, analysing data) to presenting findings in a conference-style format. This approach develops both technical skills and critical thinking, while demonstrating the connection between academic research and real-world societal impact.

The EHSS serves as a successful model for science outreach that moves beyond passive learning to active thinking. We anticipate that these motivated high school students will become effective ambassadors, discussing the environmental issues and probabilistic concepts learned with their friends, schoolmates, and family; thereby supporting our objective of communicating science to the wider community. A core achievement of the summer school was training participants to take a comprehensive and systematic approach to problems. This ability to communicate systematic thinking—not just facts—is a valuable perspective they can pass on in their discussions, helping to raise public awareness and promote more informed societal dialogue, not only about environmental challenges.

Disclaimer/Disclosure:

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6.10.T-03 Interactive Web-based Applications as Effective Data Exploration and Risk Communication Tools

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Environmental datasets are often complex mixtures of physical, geochemical, and biological data. Understanding the relationship among these groups and how they feed into risk estimates and risk assessments can be an opaque process. Challenges arise in trying to thoroughly communicate the risk assumptions and results to stakeholders that are not risk practitioners. One solution to this problem has been to present our risk assessment data using an interactive web-based platform. These applications can include data summary tables, data and risk visualizations, and geospatial analyses. This has proven to be a powerful risk communication tool for stakeholders, as the assumptions used in risk assessment (such as receptor or toxicity benchmark) can be modified and the user can instantly view how the magnitude and spatial extent of risks may change. This provides transparency to stakeholders as different exposure scenarios can be evaluated in real-time and can be used to quickly identify areas of the site or receptors that are most at-risk.

Furthermore, these applications can be built to perform and automate complex statistical and multivariate analyses, making them effective data exploration resources. This allows risk practitioners to assess and analyse the complex relationships among variables within an easy-to-understand visual interface, and is far more efficient than individually running statistical tests and data visualizations for each possible interaction.

This presentation will cover three examples where web-based tools were developed to convey complex risk assessment results to both technical and non-technical audiences. The example tools were built for a PFAS risk assessment for wildlife in a coastal system, a metals risk assessment with emphasis on bioavailability modifying factors, and a calculator for deriving media-based ecological screening values for site-specific receptors.

Web-based tools open the world of data exploration and risk communication to a broader stakeholder audience. These tools have allowed us to engage with risk assessment specialists, technologists from other disciplines, risk managers, and non-technical groups to interactively walk through complex datasets and risk assessment results. The platforms used in our examples, R and Shiny, can also be considered for incorporation into existing larger data/project communication dashboards.

6.10.T-04 Co-created Zero-pollution Solution Strategies for Micropollutant Management: Approached from a Systems Perspective

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Persistent, mobile, and potentially toxic (PM(T)) substances in the soil-sediment-water system pose a significant barrier to the reuse of natural resources and the realization of a safe circular economy in Europe. According to the European Commission, pollution by PM(T) substances is often a systemic issue, closely linked to how these chemicals are produced, used, and released into the environment. The problem is further exacerbated by inadequate technical solutions for substitution, monitoring, risk assessment and treatment within the soil-sediment-water system. Furthermore, the effective implementation of such technical solutions for managing PM(T) substances in a circular economy also depends on the surrounding social, economic, and governance conditions, referred to in this paper as boundary conditions. These conditions, as well as the associated solutions they are meant to support, vary across Europe.

This presentation presents key insights needed for collaboratively developing solution strategies to manage PM(T) substances within a circular economy. In total, four co-creation workshops focusing on three diverse circular economy routes at the local level were conducted. Our results reinforce that technical solutions alone are insufficient to achieve zero pollution without accompanying boundary conditions. Co-creation processes can help reveal these critical sticking points and bridge the gap between local needs and top-down policy design. This presentation will dive into the importance of integrating technical solutions with local governance, regulatory, and social conditions and how several of the priority actions were developed into specific policy recommendations.

6.10.P Translating Complexity – The Role Science Communication Plays in Generating Lasting Societal Impact

6.10.P-Mo420 Implications of Post-Modern, Post-Truth Philosophies and the Dunning-Kruger Effect to Micro-Nano-Plastic Environmental Risk Communication

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Science is at risk. Public trust in science has been decreasing over time and has been exacerbated by an increased rate of journal retractions over the past decade. Opinions outweigh facts, illustrating a post-modern philosophy. Confidence in decisions may be greatest by people with a little education on a topic, exceeding that of experts, evidence of the Dunning-Kruger Effect.

Scientists involved in studying the relationships between polymer chemistry and plastics understand the difficulty in educating fellow scientists and the public. Communicating the differences between various monomers, oligomers, polymers and plastics requires a deep knowledge of chemical structure and how it relates to environmental fate and potential risk. For example, there are terms used in scientific literature and social media that use the term “liquid plastics”. This term could refer to the pre-mold state of thermoset or thermoplastics. However, the term is most often used for polymers that can be dissolved in water, a violation of the definition of plastic. Polyethylene glycol is soluble, yet polyethylene (PE) plastic is not. The overlap in terminology confuses the public and decision-makers. Another example is “polyvinyl”, where polyvinyl chloride (PVC) is a well-known insoluble plastic, yet polyvinyl alcohol’s (PVA) properties are based on the degree of hydrolyzation of the polyvinyl acetate and cross linking. Properties of PVA range from soluble gels,

films and textile coatings and adhesives. The reception of such nuances by experts is often put aside by decision-makers to an “end justifies the means” perspective, i.e., post-modern, Dunning-Kruger model, potentially leading to inappropriate actions and judgements. Communications regarding plastics and similar sounding materials need to consider the broad array of properties and educational diversity. If ignored, polymers and plastics will continue to be confused and the credibility of good science dissolved.

6.10.P-Mo421 Anatomy of a Decision: The REACH Board of Appeal 2009–2025

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Since 2006, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) has reoriented chemicals governance in the European Union (EU). Within the European Chemicals Agency (ECHA), the Board of Appeal (BoA) provides an internal judicial remedy against ECHA decisions. Yet, despite more than fifteen years of decisions, no comprehensive synthesis has assessed the BoA’s overall influence or drawn lessons for the next generation of regulatory challenges. This review is therefore timely to take stock of past jurisprudence and identify improvements in anticipation of new criteria such as endocrine disruptors (ED) and persistent, mobile and toxic (PMT) substances that are expected to generate an increasing number of appeals.

We compiled and analysed all BoA decisions from 2009-2025, categorizing them by topic, outcome and stakeholder type to identify temporal trends and interpretative shifts in key REACH provisions. Descriptive statistics (annual trends, topic distribution, success rates) are combined with doctrinal analysis of pivotal rulings to trace the interpretation of key REACH provisions (information requirements, adaptations, access to studies). A thematic lens examines scientific and regulatory dimensions, including vertebrate testing as a “last resort” (article 25 of REACH regulation), weight-of-evidence assessments, read across approaches, QSAR models, and determinations of persistent, bioaccumulative, toxic (PBT) and very persistent and very bioaccumulative (vPvB) substances.

The BoA has delivered a triple contribution: (i) legal by consolidating predictability in the REACH regime; (ii) scientific by encouraging robust use of new approach methodologies and evidence integration; and (iii) regulatory by providing guidance that shapes ECHA practice and industry compliance. In addition, this review distils actionable good-practice guidance for future decision-making. By offering the first comprehensive, quantitative and qualitative synthesis of BoA jurisprudence, this work fills a notable gap and proposes a framework for measuring downstream impacts on testing practices and for tracking evolution alongside forthcoming REACH changes. The review will interest regulators, practitioners, and scientists seeking evidence-based insights at the law-science interface of chemicals policy.

6.10.P-Mo422 Daphnia Detectives

Katie Reilly¹ and Iseult Lynch², (1)*University of Birmingham, United Kingdom*, (2)*School of Geography, Earth and Environmental Sciences, University of Birmingham, United Kingdom*

Daphnia are a fantastic model organism, and widely used in ecotoxicology and environmental monitoring. Their historic use has led to a range of endpoints such as changes to life history, number of offspring, growth, movement, and molecular markers. Daphnia Detectives, is an ongoing project championing Daphnia and their ability to detect environmental issues and tell the story of these problems in an engaging way. The project is made of several strands including an introduction to Daphnia, their lifecycle, biology and use in research through microscopy. The importance of Daphnia and their ability to detect pollution, often using microplastics and fluorescent markers to engage audiences in how Daphnia can be used to detect and explore the impact of a well-known pollutant that has increasing public awareness and concern. In addition to the hands on element, Dearbhla the Daphnia (the larger than life Daphnia mascot) is a key player in the story telling and the anthropomorphising of environmental issues to share alternative perspective of environmental issues with the public.

The panel of activities and materials has been developed for showcasing at the Royal Society Summer Science exhibition in 2025 and includes highlights about generating key messages, developing hands on materials and exploring evaluation strategies to ensure that public engagement and science communication is a two-way approach. In addition, some of these materials have been further adapted to create interactive content for schools, that is freely available online and ties into the school curriculum in the UK to support STEM education and exploratory learning.

Within Daphnia Detectives we explore ethical engagement, pedagogical problem sets and strategic storytelling to demonstrate and celebrate the environmental research underway in an engaging and creative way. This work highlights ways in which researchers can adapt their research content, experimental designs and results in exciting ways to both showcase their research but also to involve different communities into the research process too to create lasting impact and legacy of engagement in STEM research and environmental impacts.

6.10.P-Mo423 From Community Collection to Data-Driven Insights: Mapping Riverine Plastic Litter with Plastic Pirates Belgium

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Plastic pollution is an issue of high priority for policymakers, since plastic litter accumulated in aquatic and land systems. Plastic litter is a source of contaminants of emerging concern (CECs), such as micro- and nanoplastics, and leached associated chemicals (e.g., additives, plasticizers, etc). Current local citizen science initiatives for litter collection and monitoring are popular and represent an opportunity to strengthen collaboration with the scientific community. Monitoring strategies have to rely on a multitude of surveying methodologies, since (plastic) litter is highly variable, and heterogeneously distributed in the environment, including in riverine and inland water systems. Citizen science initiatives can assist in monitoring, overcoming resource constraints (e.g., funding and personnel), facilitating sampling across extensive geographical areas, and increase awareness and environmental stewardship on plastic pollution in the local community. The goal of this work is to present the scientific and community engagement process of the project Plastic Pirates, using the Belgian use case as an example. This European project has been established in the Flemish region since 2022, and has engaged nearly 1500 school children, with many schools having a long-term involvement and monitoring the same geographical area over time. The resulting open access and FAIR data, which is validated by experts and data managers, is crucial for quantifying stranded litter and microplastics in riverine environments, assessing the origin and fate of plastic items, and resolving spatial-temporal litter accumulation trends in Belgium. In Flanders, the project contributes to other scientific projects such as TREASURE (Interreg North Sea) and INSPIRE (Horizon Europe), demonstrating the reach and utility of citizen science data in feeding into larger international research initiatives. Furthermore, the project includes supporting open access materials for schools and educators, available in multiple languages, and has synergies with local NGOs, actively contributing to scientific literacy and inclusion initiatives. By mobilizing the public in the scientific research process, Plastic Pirates supports research outcomes and community engagement in Belgium. All inputs from this initiative contribute to informing regulators and developing effective mitigation strategies related to plastic pollution.

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6.10.P-Mo424 Hazards in Risk Communication

Watzke de Wolf, Finland

Science communication has changed dramatically. It used to be something scientists shared mainly with other scientists—through conference talks and articles in specialist journals. Today, the goal is broader: making research understandable for the public, policymakers, and other stakeholders. Done well, it builds trust, informs decisions, counters misinformation, encourages engagement, and supports funding. Clear goals, simple language, and adapting to the audience are key. But there's another side to the story. While most discussions highlight the benefits, the downsides are often overlooked, and these can erode trust in scientists. This presentation explores the hazards associated with cultural misunderstandings and misuse of science communication. We will present findings from a survey of EU REACH regulators on national views of hazards and risks, analyzed using the Risk Perception Attitude (RPA) framework. We'll also show examples of risk communication used for economic gain—by companies for commercial purposes or by scientists and research groups to increase the chances of their proposals being funded. The speed of online communication makes the challenge to avoid miscommunication and misuse even greater. Scientists need to work with social scientists and professional communicators to craft messages that are clear, honest about uncertainties, and sensitive to cultural differences in today's digital world.

6.11.P Translating Laboratory-Based Ecotoxicological Assessments to Population-Level Effects of Chemicals

6.11.P-Th275 What('s in) the shell! Is shell deposition an acute endpoint?

Sven Ritschar¹, Alan J. Jones², Maike Habekost¹ and Lennart Weltje³, (1)BASF, Germany, (2)BASF, United States, (3)Agricultural Solutions - Ecotoxicology, BASF, Germany

Regulatory ecotoxicology increasingly uses endpoints beyond survival to capture species-specific sensitivities. The oyster shell deposition test (OCSPP 850.1025) measures shell growth inhibition as an acute toxicity indicator, yet it is often treated as a survival endpoint in European risk assessments. This misclassification overlooks that shell deposition reflects metabolic processes rather than lethality, leading to overly conservative risk characterizations and disproportionate refinement steps without improving ecological relevance. In contrast, U.S. regulatory frameworks explicitly recognize this endpoint as an acute growth measure, underscoring a more proportionate approach compared to the conservative EU.

Further, there is evidence that oysters are less, or similarly, sensitive compared to standard aquatic species when exposed to pesticides. Therefore, a comparison of growth and survival endpoints of the eastern oyster was conducted and compared to survival endpoints for *Daphnia magna*. Results indicate that the use of the growth endpoint in environmental risk assessment is not appropriate, particularly for acute risk assessment where survival-based endpoints are more biologically meaningful. While mollusc endpoints add value by broadening species coverage, their interpretation must align with biological meaning to avoid disproportionate outcomes. Clear distinction between growth-based and survival endpoints will ensure scientifically sound decisions and maintain proportionality in risk management.

6.11.P-Th276 Progressing Short-Term Methods to Estimate Chronic Marine Toxicity for Regulatory Use in the North-East Atlantic Region

Kirit Wadhia¹ and Mathijs Smit², (1)National Oilwell Varco (NOV), United Kingdom, (2)Shell Global Solutions International BV, Netherlands

1. 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.

2. 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.

3. 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.

4. Implementation Plan

- Review and analysis of existing protocols
- Protocol selection and testing plan design
- Pilot testing
- Dissemination

6.11.P-Th277 Integrating Behavioural Endpoints and Community-Level Effects to Understand Ecosystem-Level Impacts of Psychopharmaceuticals

Elieen Versteegen, Edwin Peeters, Ivo Roessink and Paul van den Brink, Wageningen University and Research (WUR), Netherlands

Aquatic environments are increasingly contaminated with psychopharmaceuticals, raising concerns about their impacts on non-target organisms. Compounds such as carbamazepine (CBZ) and citalopram (CIT) may interact

with organisms' neuroendocrine systems, altering their behaviour. Yet, the ecological relevance of behavioural changes under realistic exposure conditions remains uncertain. To investigate this, we combined behaviour-oriented 48h laboratory experiments with 13-week outdoor mesocosm studies to assess behavioural, population-, and ecosystem-level effects of CBZ and CIT.

In laboratory assays, movement-based behavioural endpoints in *Gammarus pulex* were more sensitive to CBZ and CIT than mortality, showcasing their potential as sensitive indicators of pharmaceutical exposure. However, behavioural effects of CBZ and CIT occurred only at concentrations far above those found in the environment, suggesting that short-term exposure is unlikely to trigger responses in the environment.

Because environmental exposure is typically chronic, we conducted two mesocosm studies to examine the long-term effects of CBZ and CIT on the behaviour of *G. pulex* and *Lymnaea stagnalis*, as well as on the broader aquatic community. The exposures had little effect on *G. pulex* movement but caused noticeable changes in *L. stagnalis* locomotion. These behavioural alterations did not translate into measurable population-level effects. After 13 weeks of exposure, no significant effects were detected on water quality, bacterial composition, zooplankton, macroinvertebrates, or community structure, indicating that environmentally relevant concentrations up to 100 µg/L are unlikely to produce population- or ecosystem-level impacts under these conditions. These results highlight the complexity of linking behavioural responses to ecological outcomes. Although behavioural endpoints show promise as early indicators of pharmaceutical exposure, their ecological relevance and suitability for regulatory risk assessment remain uncertain. Future research should focus on long-term studies which include effects on reproduction, as short-term behavioural changes may not translate into population-level effects, and organisms may adapt over time. Mesocosm studies offer a valuable addition to single-species tests by capturing species interactions, community dynamics, and indirect effects, providing a more realistic foundation for assessing the ecological risks of psychopharmaceuticals.

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6.11.P-Th278 The SETAC MAPPED workshop: a proposed list of fish species to assess for the population relevance of endocrine disruption

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Agrochemicals and biocides require an assessment on whether they have endocrine disrupting properties. A substance is considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms when it meets specific criteria. However, according to the definition in Commission Regulation (EU) No 2018/605, this classification applies "unless there is evidence demonstrating that the adverse effects identified are not relevant at the (sub)population level for non-target organisms," which aligns with the protection goals of ecotoxicological risk assessments. Currently methods to consider population relevance of endocrine disruptors have not been available, so a SETAC workshop on the use of 'Models to Assess the PoPulation relevance of effects of Endocrine Disruptors (EDs)' (MAPPED) was formed to explore the issues for fish and amphibians.

To assess whether the effects of ED are relevant at population level, a key pre-requisite is to understand the population biology of the affected fish species. To ensure protection for all fish species in Europe, the population relevance assessment should focus on the most vulnerable species. Here a vulnerable species is defined as a species that is innately sensitive, likely exposed to the chemical(s), and has a life strategy or behaviour that may make it less resilient. Hazard assessment for ED assumes that all species are exposed and sensitive. Therefore, our approach to species selection has focused on life-strategy and behaviour. In this presentation, we identify key traits for life-history and behaviours most relevant for assessment of effects from

EDs. All freshwater fish species in Europe with sufficient information on relevant traits were clustered based on their trait combinations, and representative species for each cluster were then chosen. To perform a fully comprehensive assessment of the potential hazard to all European fish populations from ED under the current regulatory regime would require an assessment for all species relevant for the effect selected from all clusters. As such, this first proposal was to identify a set of focal fish species to assess population relevance of ED to help guide efforts in developing suitable population models.

Disclaimer/Disclosure:

The presented work was conducted at the SETAC MAPPED workshop

6.11.P-Th279 The SETAC MAPPED workshop: Application of effect modelling to assess population relevance of endocrine disruption mediated effects on freshwater fish

Melissa Reed¹, Udo Hommen², Charles Hazlerigg³, Kate Mintram⁴, Erik B Muller⁵, Charles Tyler⁶ and Thomas G. Preuss⁷, (1)Health and Safety Executive (HSE) - Chemical Regulation Division, United Kingdom, (2)Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Germany, (3)Enviresearch Ltd., United Kingdom, (4)Brunel University, United Kingdom, (5)ibacon GmbH, Germany, (6)University of Exeter, United Kingdom, (7)Bayer Research & Development Services LLC, Germany

Plant protection products and biocides require an assessment of whether they have endocrine disrupting properties. In EU Regulation 2018/605, however, there is a caveat to the definition of endocrine disrupting properties, whereby it states - “unless there is evidence demonstrating that the adverse effects identified are not relevant at the (sub)population level for non-target organisms”. Currently methods to consider population relevance have not been available, so a SETAC workshop on the use of ‘Models to Assess the PoPulation relevance of effects of Endocrine Disruptors (ED)’ (MAPPED) was formed to explore the issues for fish and amphibians.

The working group on fish populations has explored the selection of species to be modelled, processes they believe should be included in a model that assesses consequences of endocrine disruption on populations, what is needed for an environmental scenario, how to assess population relevance from the model output and the validation process for the models. For all these topics, the focus is on how they differ when addressing the hazard of endocrine disruption rather than using a population model for risk assessment.

A case study is presented that shows how a population model may be used for this assessment. For this some issues specific to a hazard assessment (e.g. how do you set up a model when the level and pattern of exposure are not be taken into account) and some issues are related to population level assessment in general (e.g. how to account for variability and uncertainty when deciding if a difference to control runs indicates an adverse effect on the population).

The case study uses an individual based three-spined stickleback model to explore these issues. Two example endocrine disrupting chemicals are used to illustrate how effects can be extrapolated from typical regulatory studies to population level effects. The chemicals selected have been chosen because they are known EDs with different effects at the organism level and can be used to explore how these effects may, or may not, translate to population level effects.

Track 7: Moving Beyond - Cross Cutting Themes, Emerging and Transdisciplinary Topics

7.01 Advances in Predictive and Regulatory Toxicology and Ecotoxicology: Issues, Challenges and Opportunities

7.01.T-01 Building nano-QSARs and the importance of assessing their limitations

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The toxicity of engineered nanomaterials (ENMs) can be altered depending on their physico-chemical properties or environmental conditions, which makes toxicity testing time-consuming and costly. NanoQSARs can be effective here to aid in the risk assessment of ENMs. Quantitative structure activity relationships (QSARs) are built under the assumption that structurally similar substances will result in similar biological effects.

Nano-QSARs can be built using various different machine learning algorithms that can rapidly learn from large quantities of complex data. While machine learning algorithms have powerful predictive capabilities, it is important that the limitations and biases of nano-QSARs are properly evaluated to ensure robust and accurate predictions. This study investigates model limitations and prediction uncertainties of nano-QSARs in depth and discusses the importance of proper model assessment.

7.01.T-02 Mechanistic Model for Predicting Acute Oral Toxicity: Data Uncertainty and Model Predictability

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Everyday exposure to various chemical compounds with potential adverse effects on human health, combined with the regulatory ban on using animals in acute oral toxicity (AOT) testing for cosmetic ingredients and products, are prerequisites for developing reliable in silico models. A read-across (RA) workflow based on mechanistic considerations for predicting AOT has been built in the QSAR Toolbox using a training set of 10,069 chemicals. The model automatically provides a numerical rat oral LD50 value for AOT and a categorical prediction in the form of a GHS classification distribution. The training set is initially divided into groups (scenarios) of chemicals that share the same protein binding reactivity and/or specific mechanisms related to acute oral toxicity (e.g., mitochondrial uncoupling). The model also accounts for in vivo metabolic activation and the neutralization of parent chemicals. When an untested chemical is submitted for prediction, the model automatically identifies its reactivity mechanism and assigns it to the relevant scenario associated with that mechanism. This process forms a primary group of mechanistically similar analogues. The primary group is then refined to include the most structurally similar compounds by applying rule-based structural profiling in the QSAR Toolbox. The LD50 prediction for the target chemical is based on a RA with analogues that are both mechanistically and structurally similar. The model's numerical performance achieved an R2 of 0.89. The reliability of the predictions is evaluated through a three-layered applicability domain. The accuracy of both numerical and categorical predictions is assessed, considering the uncertainty in experimental data and the limitations of the modeling approach. Experimental uncertainty defines the probability distribution of the numerical prediction, i.e., the confidence interval, which takes the form of a Gaussian distribution. This Gaussian distribution also informs the GHS classification prediction. Two extremes are possible: an unambiguous GHS classification, where the distribution is sharp and the probability of falling into a single category is high; and an ambiguous classification, where the distribution is broad and no definitive GHS category can be assigned. The model's GHS categorical performance was found to be an R2 of 0.91.

7.01.T-03 Performance of models predicting ready biodegradability

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ECHA's guidance states that (Q)SAR models for predicting ready biodegradation are generally not yet accurate enough to reliably predict rapid degradation. (Q)SAR predictions can, on the other hand, be used as supporting evidence to indicate that the substance is not readily biodegradable. In this presentation, the results of curation of REACH experimental data on ready biodegradation and use of this curated dataset for the unbiased external validation of various (Q)SAR models, including Biowin, Opera, Vega, Catalogic, and by Körner et al.(2024), will be presented.

The extracted REACH data were curated to remove non-experimental data, unreliable experimental studies, studies with test material other than discrete organics, studies failing guideline validity criteria and substances with conflicting results. The curated dataset of experimental data, including 2684 structures (691 labelled as readily biodegradable and 1993 as non-readily biodegradable), not present in the training and validation sets of the targeted models allowed for their unbiased external validation. The models were compared based on how well their training sets span the industrial chemical space, their predictive performance and applicability domain coverage.

The balanced accuracy ranged from 0.600 to 0.771, while the sensitivity for identifying non-readily biodegradable substances varied between 0.217 and 0.848. The applicability domain coverage ranged from 28.5% to nearly the entire chemical space, yet the predictive performance remained relatively unaffected even for structures outside this domain. Further analysis in regard of precision and negative predicted value indicating the proportion of predicted readily biodegradables which have been also experimentally tested as readily biodegradable, indicated that "readily biodegradable" predictions may still not be accurate enough to reliably

predict rapid degradation while the chances of a correct non-readily biodegradable prediction are much higher. Based on the global performance results, consensus modelling offers limited advantages for general use compared to using Biowin 6 alone. However, it can be valuable when the goal is to identify a small number of highly certain positives or negatives, or to increase confidence in the predicted biodegradability of a single substance. This could make consensus modelling a promising approach for replacing an experimental test, but it comes at the detriment of a reduced applicability domain.

Disclaimer/Disclosure:

The above represents the opinion of the authors and is not an official position of the European Chemicals Agency.

7.01.T-04 From Literature to Mechanistic Insight: A Retrieval-Augmented Generation-Driven Framework for Nanomaterial Adverse Outcome Pathways

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The extensive use of nanomaterials (NMs) has led to increasing releases into aquatic environments, raising growing concern about their potential ecotoxicity. Due to their unique physicochemical properties, NMs can interact with aquatic organisms in complex ways and induce various hazardous effects. Traditional experimental approaches are time-consuming, costly, and unable to cover diverse species, highlighting the need for an efficient and economical ecological risk assessment framework to elucidate the toxicity mechanisms of nanomaterials. The Adverse Outcome Pathway (AOP) framework provides a structured approach to toxicity evaluation by linking molecular initiating events (MIEs) triggered by NMs to subsequent key events (KEs) across biological levels and ultimately to adverse outcomes (AOs) relevant to risk assessment. However, the highly complex bio-environmental interactions of NMs and the requirement to integrate scattered literature, multidisciplinary knowledge, and multi-level evidence make manual AOP construction labor-intensive and prone to missing critical information. Large Language Models (LLMs) offer new opportunities for advancing NMs AOP development. With strong abilities in extracting information from vast literature, integrating cross-domain knowledge, and identifying complex biological relationships, LLMs can substantially reduce the time required for data synthesis and help overcome gaps in expert knowledge, thereby improving the depth, coherence, and accuracy of AOP construction. However, the knowledge depth of the general large model in the field of AOP in nanomaterials is not yet sufficient to assist us in AOP development. In this study, we focused on nanoparticles and developed an AOP knowledge base through a systematic review of nanotoxicology literature. By incorporating Retrieval-Augmented Generation (RAG), we equipped an LLM with expert-level knowledge in nanoparticle AOPs and built a specialized question-answering system for nanomaterial AOP analysis. Our results show that the RAG-enhanced LLM significantly outperforms a general LLM in answering NMs AOP-related queries. It not only improves understanding of nanoparticle toxicity mechanisms but also demonstrates strong potential to support the development of comprehensive AOP networks for NMs.

7.01.T-05 From Machine Learning to Fundamental AI Models in QSAR: How to Improve the Regulatory Acceptance?

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Quantitative structure-activity relationship (QSAR) models remain one of the most prominent *in silico* New Approach Methods (NAMs). The OECD QSAR Assessment Framework (QAF) can be viewed as a comprehensive procedure for evaluating both existing and newly developed QSAR models. However, an open question is to what extent this framework reflects the most recent advances in the field — particularly the emergence and application of fundamental AI models. Furthermore, does a positive QAF evaluation necessarily ensure regulatory acceptance? And what is the role of human judgment in this process, and how can its impact be minimized?

In this contribution, we discuss several additional factors that, in our view, should be considered when evaluating the regulatory acceptability of QSAR models: (i) the toxicological relevance of the model, (ii) regulatory relevance of the model, and (ii) its user-friendliness and intuitiveness. We then present a case study comparing the outcomes of applying QAF – together with these two supplementary criteria – to predictions of

the same 3 endpoints generated by a classical machine-learning QSAR model and by a QSAR model based on a fine-tuned FM4M fundamental AI model recently developed by IBM. We hope that our analysis will contribute meaningfully to the ongoing discussion and offer valuable guidance for developers of AI-driven QSAR tools seeking to maximize their potential for regulatory acceptance.

7.01.P Advances in Predictive and Regulatory Toxicology and Ecotoxicology: Issues, Challenges and Opportunities

7.01.P-We422 Ensuring Regulatory Compliance with the (Q)SAR Assessment Framework: Best Practices and Case Examples of Reporting (Q)SAR Predictions for REACH

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QSAR models are an efficient, cost-effective alternative to assess the hazards of chemicals for regulatory purposes. By promoting the application of QSARs, ECHA aligns with the ethical concerns associated with animal testing (the principles of the 3Rs: Replacement, Reduction, and Refinement) and aims at enhancing the scientific robustness of chemical assessment. On the other hand, ECHA's efforts in protecting health and the environment require the careful assessment of how computational models are used to ensure an adequate level of protection.

The (Q)SAR Assessment Framework (QAF), developed by ECHA in collaboration with the Italian National Institute of Health (ISS) and the Organisation for Economic Cooperation and Development (OECD), represents a significant advancement in the field. It provides a consistent and transparent methodology for regulators, users and developers to evaluate the reliability and relevance of (Q)SAR models and predictions. The QAF includes a series of assessment elements and detailed checklists for assessing models, predictions, and results from multiple predictions, ensuring clarity, consistency, and transparency in the evaluation process. Hence, (Q)SAR users are informed of the requirements and can enhance their application and documentation, increasing the probability of regulatory acceptance. Furthermore, the framework encourages (Q)SAR model developers to create models that are fit for regulatory applications, thereby increasing confidence in the use of these models for hazard assessment.

The QAF is meant to be broadly applicable irrespective of the modelling technique, the predicted endpoint, and the intended regulatory purpose, and therefore does not provide exhaustive instructions on all possible aspects. There is a need to adapt it to specific regulatory applications, and it provides certain flexibility with regards to interpretation. In this presentation, we will provide case examples and recommendations about the best practices to report (Q)SAR predictions for facilitate REACH compliance on basis to the QAF.

7.01.P-We423 Strengthening Regulatory QSAR Evaluation: Application of the QSAR Assessment Framework to a Bioaccumulation Case Study

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The growing use of in silico Quantitative Structure-Activity Relationship (QSAR) methods offers significant opportunities to strengthen regulatory chemical assessments while reducing animal testing. However, the consistent and transparent evaluation of QSAR predictions remains a key challenge for regulatory acceptance. The QSAR Assessment Framework (QAF) was developed to provide practical, harmonized guidance for evaluating QSAR models and predictions across different regulatory contexts. Building on internationally recognized principles, such as the OECD QSAR validation criteria, the framework supports assessors and registrants in judging model reliability, assessing applicability domains, and integrating predictions within a weight-of-evidence approach.

This work outlines the core elements of the QAF and demonstrates its application in the context of REACH registration from the user point of view. It was selected a case study that focuses on fulfilling a specific REACH information requirement for bioaccumulation and illustrates how the framework supports evaluation of QSAR predictions. It shows how all requirements defined in the QAF are addressed to justify a QSAR prediction, with particular emphasis on evaluating prediction reliability and uncertainty. This includes the selection of appropriate analogue chemicals to support endpoint predictions, the use of metabolism simulation, and the analysis of applicability-domain outcomes. The results are structured using the (Q)SAR Model Reporting Format (QMRF) and (Q)SAR Prediction Reporting Format (QPRF), in full alignment with the requirements

outlined in the QAF document. The automated population of the QPRF file with information required for the QAF assessment elements as well as the possibility further direct transferring to IUCLID is an essential part of reporting process that will benefit both – facilitation of the users in report preparation and consistent evaluation by the regulators.

7.01.P-We424 Performance Of the iSafeRat® Model in the Recent ECHA Evaluation of Chronic Fish Toxicity Predictions

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In the context of regulatory ecotoxicological assessment under REACH and the need to limit animal use in experimental testing, ready access to reliable QSAR predictions for chronic fish toxicity has become increasingly important. Recently, ECHA published a comprehensive analysis entitled “Replacing Fish Early Life Stage Toxicity Test: Performance of Three QSAR Tools for Recently Submitted REACH Data”, evaluating the predictive performance of three models—ECOSAR, VEGA and iSafeRat®—against newly submitted experimental datasets. This poster focuses on the results obtained for the iSafeRat® chronic fish toxicity model and discusses the specific issues associated with our predictions and lessons learned for future model refinement at KREATiS. The ECHA evaluation was conducted on a dataset of 49 substances for which iSafeRat produced predictions for 18 compounds falling within its applicability domain, consistent with its principle of refusing to extrapolate beyond domain boundaries. Out of these 18 predictions, 17 were classified by ECHA as well-predicted, while one was considered an outlier. Overall, we are satisfied with these results, especially considering that the ECHA evaluation method corresponds to a worst-case scenario as toxicity predictions were generated directly from SMILES with no refinement or correction of intermediate parameters. The standard KREATiS regulatory workflow relies on a three-step QSAR cascade: prediction of log KOW from structure, prediction of water solubility from log KOW, and prediction of chronic fish toxicity from solubility. For QPRFs prepared by KREATiS, each intermediate value is systematically checked against reliable available experimental data to ensure coherence and to correct upstream parameters when necessary. Our analysis of the ECHA evaluation shows that the single poorly predicted substance exhibited an inaccurately predicted water solubility value. When replacing the predicted water solubility with the corresponding experimental value, the resulting chronic fish prediction becomes consistent with the experimental toxicity. This observation highlights not only the importance of scrutinizing and validating intermediate properties, but also how model users can optimize predictive accuracy through informed parameter correction. With ongoing refinements, we anticipate enhanced performance in forthcoming evaluations, exceeding what was observed under the stringent conditions of the recent ECHA publication.

7.01.P-We425 How to: Integration of Quantitative Structure Activity Relationships into the Safe and Sustainable by Design Framework

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The Safe and Sustainable by Design (SSbD) framework was introduced in the European Commission (EC) within the Chemicals Strategy for Sustainability (CSS) which promoted innovation for safe and sustainable chemicals toward an environment free of toxic substances. This framework is currently a voluntary approach to guide the innovation process for chemicals and materials. SSbD integrates an assessment of the environmental sustainability with safety considerations to provide a comprehensive evaluation. The general structure of the SSbD framework is composed of two phases, a first one for the innovation (redesign) of a chemical/material, and a second one for safety and sustainability assessment. The EC's SSbD framework supports and promotes, especially in the early stages of innovation, the use of new approaches to test and evaluate chemicals and materials, including predictive tools, which sometimes may involve a higher degree of uncertainty. This work explores the integration of in silico approaches within SSbD and how strategies based on quantitative structure–activity relationships (QSARs) can estimate missing data for properties considered in different steps of the framework, for existing and new chemicals, in line with Regulation (EU) 2024/1781 on Ecodesign for Sustainable Products (ESPR). The proposed workflow addresses parameters listed in Annex I of the ESPR Regulation—such as substances of concern, product environmental (PEF) and carbon footprint (PCF), and durability—and outlines how QSAR-derived data can be incorporated into the Digital Product Passport for traceability and compliance.

The workflow tackles key challenges: identifying stages where QSARs add value; integrating data while ensuring reproducibility according to OECD principles; assessing and communicating uncertainty transparently;

and harmonising outputs from multiple QSAR tools to meet regulatory requirements for verifiability and standardisation. It also considers how computational methods can reduce testing costs and administrative burden, supporting Small and Medium-sized Enterprises (SMEs) and accelerating innovation. In conclusion, this work proposes a systematic approach to select and integrate QSAR models within SSbD, ensuring alignment with EU sustainability and ecodesign requirements, facilitating digital information management, and promoting harmonisation for future regulatory frameworks.

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7.01.P-We426 Potentials and challenges for mobility assessment of UVCB substances using in silico tools *Antje Gerloff-Elias and Claudia Ileana Cappelli, knoell Germany GmbH, Germany*

The assessment of substance mobility in soil has become increasingly important with the introduction of “new” hazard classes under Regulation (EC) No 1272/2008 (Classification, Labelling and Packaging of substances and mixtures; CLP) for persistent substances.

Results from OECD 106 and 121 studies can provide crucial data for assessing the potential mobility of a substance. However, experimental normalised organic carbon to water partition coefficient (KOC) data (the primary parameter for mobility assessment) is available for only approximately 20% of EU REACH-registered substances, creating significant data gaps. Furthermore, adsorption testing is often time-consuming, expensive, and difficult to scale for broad assessments, particularly for complex scenarios or many substances. Specifically for difficult to test substances such as instable substances or substances of unknown and variable composition (UVCBs) or ionisable substances, the experimental design is challenging.

In silico predictions offer an alternative to assess the mobility of substances, when no experimental data is available. Opportunities include high-throughput analysis of complex chemical mixtures and facilitation of prioritisation of substances/constituents for further testing. Limitations stem from the variable composition of UVCBs making accurate modelling difficult, the uncertainty of model predictions, and the need to bridge gaps between in silico output and real-world experimental validation, particularly for regulatory compliance. Here we describe the challenges for mobility assessment of complex UVCB substances and present a workflow how in silico-generated predictions can support mobility assessment under CLP and EU REACH.

7.01.P-We427 Simulating Kinetics of Metabolism. QSAR Application

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The importance of toxicokinetics in predicting toxicological endpoints has increased markedly over the years. It has evolved from being completely disregarded in early models to being modelled qualitatively and later quantitatively. The Laboratory of Mathematical Chemistry (LMC) has extensive experience leveraging toxicokinetics to model various effects. Initially, a large set of tissue-specific metabolic simulators was developed and continuously improved by incorporating additional documented metabolism data and expert knowledge. These simulators enable the generation of potential metabolites for any target and the subsequent application of hazard alerts responsible for the ultimate prediction. Later, an attempt was made to quantify hazard potential by calculating the amounts of active metabolites (i.e., those causing effects such as protein adducts). In recent developments, we focus on modelling the kinetics of metabolism, aiming to explicitly express the quantities of parent chemicals and their metabolites as functions of time. Practically, this task is achieved by developing a novel model to predict reaction rates. Due to data deficiencies and insufficient metabolic information, the present work considers in vitro (rat) half-lives of parent structures to indirectly estimate reaction rates. To reduce misclassification caused by various possible transformations, here we focus on two reaction types: hydrolysis and hydroxylation. We examined various chemical parameters (2D, 3D, local) as potential descriptors. The reliability of the predictions is evaluated through internal cross-validation and has proven valuable. However, the results should be considered preliminary due to the limited scope of modelled transformations and the small size of the training dataset. Future model developments are planned and will

involve expanding the training set with additional transformation types, as well as other species (e.g., humans) and tissues (e.g., in vivo half-lives).

7.01.P-We428 Validation of machine learning-based QSAR models using reference chemicals for regulatory applications

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Machine learning-based QSAR models were developed to predict regulatory-relevant toxicological endpoints, including acute and chronic toxicity, carcinogenicity/mutagenicity/reproductive toxicity (CMR), and neurotoxicity, by integrating large-scale in vitro assay data with curated in vivo toxicity information. The models were constructed to capture mechanistic signals associated with adverse outcomes, enabling transparent linkage between assay-derived biological activity patterns and apical toxicity endpoints of regulatory interest. To evaluate their robustness, applicability, and regulatory readiness, the models were validated using well-established reference chemicals provided by international bodies involved in NAMs evaluation, including ECVAM and other validation-oriented agencies. These reference chemicals span diverse chemical classes and toxicity modes of action, providing a rigorous basis for assessing model performance across different domains of applicability. Comparative analysis demonstrated that the models achieved consistent predictive accuracy across endpoints, and mechanistic concordance analyses indicated that predictions were supported by biologically plausible assay profiles. The results collectively highlight that ML-based QSAR models, when anchored to empirical in vitro and in vivo data and evaluated with internationally recognized reference chemicals, can offer reliable support for regulatory decision-making. This study underscores the potential of NAMs-driven computational approaches to enhance hazard assessment frameworks and contribute to the broader acceptance of AI-enabled predictive tools in regulatory toxicology.

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7.01.P-We429 Improving the predictive performance of toxicity prediction machine-learning models using state-of-the-art deep learning approaches

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In our previous study, machine learning models were developed to predict regulatory-relevant toxicological endpoints using curated in vitro and in vivo datasets; however, for several key endpoints, predictive performance remained limited due to small dataset sizes and highly imbalanced class distributions. These constraints hindered the ability of conventional machine learning algorithms to learn meaningful decision boundaries and capture mechanistic signals associated with adverse outcomes. To address these challenges, we explored the applicability of state-of-the-art deep learning approaches increasingly adopted in the computer science field, including advanced data augmentation strategies, representation learning, and architectures optimized for learning from sparse and imbalanced data. By integrating these methods into the existing toxicity prediction workflow, we systematically evaluated whether modern deep learning techniques could enhance model robustness, improve classification performance, and expand the domain of applicability for endpoints historically constrained by data limitations. Preliminary results demonstrate notable performance gains for selected regulatory endpoints, suggesting that next-generation deep learning methods can complement traditional machine learning models and offer a viable pathway for improving toxicity prediction when empirical data are scarce or unevenly distributed. This study highlights the potential of leveraging cutting-edge computational techniques to advance predictive toxicology and strengthen model readiness for regulatory applications.

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7.01.P-We430 Comparison of OECD 236 Guideline with the Toxicity Profile of Zebrafish Cell Lines
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The OECD 236 guideline provides an alternative to animal testing for acute fish toxicity, as required by REACH, BPR, and PPP in Europe. Although this assay reduces the use of animals, it involves zebrafish embryos, which are not regarded as fish under regulatory definitions because they are not considered independently feeding larval forms; however, they are still whole living organisms. To further reduce animal use, OECD 249 was developed as a fully in vitro method using rainbow trout gill cells (RTgill-W1) as a substitute for OECD 236 and 203; however, its regulatory acceptance remains limited.

In this study, we aim to determine whether essays conducted under OECD 236 produce toxicological data comparable to those from an adaptation of OECD 249 using zebrafish cell lines instead of rainbow trout gill cells. To achieve this, we have developed a Quantitative Structure-Activity Relationship (QSAR) model trained on curated acute toxicity data from OECD 236 studies and compared its predictions with experimental results obtained from acute toxicity assays using zebrafish embryo cell lines. These findings may help advance regulatory adoption of zebrafish-based in vitro assays aligned with OECD 249 principles, thereby reducing reliance on animal experimentation and promoting next-generation risk assessment strategies.

7.01.P-We431 The role of chemical specificity in modelling approaches

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At present, over 350,000 chemicals are registered for production and use worldwide. These registered substances enable a wide range of industrial and societal applications, but also pose risks to human health and the environment. To manage chemical safety, regulatory frameworks rely on hazard and risk assessments. However, these assessments face significant challenges, including i) a lack of reliable and comprehensive data, and ii) a relatively slow and inefficient evaluation and regulation process.

Innovation of chemicals evolves rapidly, with estimates of over 2,000 new chemicals introduced to the global market yearly. These innovations are increasingly driven by chemical specificity, particularly in the context of mode of action (MoA). Yet most predictive models in toxicology and risk assessment are grounded in data-driven patterns. This creates a fundamental friction: the more specific a MoA is, the less detectible these data-driven patterns become during modeling, as they are overshadowed by patterns of the larger data mass.

Quantitative Structure Activity Relationships (QSARs) are created based on the structural information of chemicals with the aim of applying them to novel (untested) substances. However, from (Balraadjsing et al., 2024) and Minnema et al. (submitted) we learned that QSARs do not always generalize well when applied to novel materials or chemicals with specific MoA. Here we build upon this idea to describe a framework that investigates how chemical specificity can be better acknowledged in modelling approaches.

7.02 Advancing Pollution Control in Wastewater: Monitoring, Risks, and Regulatory Transitions

7.02.T-01 Get to know the European HORIZON Project URBAN M20 “Effective Monitoring and Modelling solutions for data-driven holistic management of urban water quality”

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The European Union’s Zero Pollution Action Plan sets ambitious targets for cleaner urban water environments, aiming to reduce health risks and environmental impacts caused by stressors such as chemical and microplastic emissions. Achieving these goals requires, first, extensive high-quality data; and second, robust support for pollution control strategies throughout the entire urban water cycle. However, the current Best Available Monitoring Methods (BAMMs) face critical limitations. Laboratory analyses and sensor technologies often target only a small range of contaminants. They also involve high operational costs and require complex maintenance. Furthermore, they provide data with limited spatial and temporal resolution, which poses challenges for their broad application by water utilities. Although digital twins and modelling tools can help to bridge these gaps, they rarely integrate real-time data and are subject to significant uncertainties. Furthermore,

fragmented data storage across institutions restricts accessibility for decision makers.

The HORIZON Europe and SERI funded URBAN M2O project, launched in June 2025, addresses these challenges by developing integrated solutions, such as:

- a. combining validated, AI-enhanced monitoring sensors with fit-for-purpose modelling tools tailored to the needs of regulators and water utilities and benchmarked against BAMMs (organic chemicals, metals, microplastics, pathogens, and effect-based tools);
- b. establishing a multistakeholder Data Management System (DMS) to deliver 'whole system' and open access information, enabling a harmonised approach to urban water quality management;
- c. creating a flexible Digital Urban Water Twin (DUWT) to simulate pollutant pathways and evaluate reduction strategies in different climate scenarios. These will be demonstrated in three EU case studies, which will showcase varying data availability and climate challenges.

The URBAN M2O project involves end users, industrial partners, and academic institutions to ensure co-production of knowledge from design to implementation. The project invites sensor developers and interested urban communities to join as external partners, offering them the opportunity to test their sensors against comprehensive BAMB measurements or explore the digital urban water twin for their own water cycles.

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7.02.T-02 Chemical Target Screening and Mixture Risk Assessment of Wastewater: A Pan-European Study

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Complex mixtures of chemicals from domestic and industrial activities reach the aquatic environment via Wastewater Treatment Plant (WWTP) effluents. These micropollutants are often only partially removed by conventional treatment processes, contributing to toxic pressure on aquatic organisms. The revised Urban Wastewater Treatment Directive (UWWTD) emphasises priority substances and improved monitoring, yet harmonised large-scale mixture assessment remains limited. Thus, in this study, influent and effluent samples from 30 WWTPs across 15 European countries were investigated to: (1) better understand chemical occurrence, (2) evaluate removal efficiencies, (3) assess the risks posed by complex chemical mixtures on key biological quality elements, and (4) examine how well the UWWTD benchmark substances represent broader mixture behaviour. Chemical target screening resulted in 402 compounds reliably detected and quantified, with human pharmaceuticals representing the largest group of detected compounds. Concentrations ranged from <1 ng/L to >200 µg/L, and only 44% of compounds were well removed (>80 removal) by conventional treatments, while most exhibited limited removal. Mixture risk assessment revealed widespread exceedance of chronic toxicity thresholds across Europe, particularly for algae and daphnia. Based on compounds with available measured effect data, the main risk drivers for algae were ketoprofen and N,N-Dimethyldodecylamine, while for daphnia, risks were dominated by fipronil and diazinon. Under a no-dilution scenario of the effluent, chronic risk thresholds were exceeded at 60% of the sites for algae and in all sites for daphnia. UWWTD benchmark substances were detected in over 94% of effluents, confirming their limited treatability under conventional treatments. Based on average ozonation removal rates from the literature, predicted quaternary treatment scenarios indicated substantial reductions of effluent concentrations and risks. Overall, micropollutants were widespread and often poorly removed by conventional treatment. Mixture risks were largely driven by non-benchmark substances, and although predicted quaternary treatments reduced the concentrations and toxic pressure, their effectiveness is not uniform across chemical mixtures and biological quality elements. This highlights the need for widespread mixture-based monitoring and expanded benchmarks to ensure protection of aquatic ecosystems.

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7.02.T-03 Effect-Based Monitoring Identifies Macrolide Antibiotics as Main Toxicity Drivers in Flemish Wastewater Treatment Plant Effluents, Belgium

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The revised EU Urban Wastewater Treatment Directive (UWWTD) incorporates new obligations for advanced treatment and enhanced monitoring of micropollutants in urban wastewater systems, supporting more robust strategies for protecting surface-water quality. However, chemical monitoring alone often fails to capture mixture effects relevant to ecological health. This study evaluated the ecological risks of organic micropollutants (OMPs) mixtures in effluents from 16 wastewater treatment plants (WWTPs) across Flanders, Belgium, by integrating effect-based monitoring (EBM) with targeted chemical analysis. Bioactivity of solid-phase extraction (SPE) effluent extracts was assessed using a *Microcystis aeruginosa* growth inhibition assay. Among 130 identified compounds, 26 OMPs were quantified across major classes. The bioassay showed high sensitivity, with EC10 and EC50 values corresponding to relative enrichment factors of 1.1–4.7 and 4.1–38, respectively. Iceberg modeling indicated that macrolide antibiotics, particularly azithromycin and clarithromycin, accounted for most of the observed inhibitory effects, while remaining bioactivity likely reflects contributions from other, unquantified mixture components. Risk characterization in receiving waters using chemical risk quotients, effect-based trigger values, and margin-of-safety metrics consistently identified potential ecological concern ($RQ > 1$) at most sites, with smaller WWTPs and low-dilution catchments exhibiting the highest risk. This work demonstrates the added value of combining EBM with chemical monitoring to improve mixture risk assessment, identify key toxicity drivers, and support regulatory transitions toward more holistic effluent evaluation under the UWWTD.

Disclaimer/Disclosure:

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7.02.T-04 Volatile organic compounds as early indicators of bleach and antibiotic stress in anaerobic microbial communities

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Anaerobic digesters and septic tanks depend on stable microbial communities, yet early detection of disturbance remains difficult because routine indicators such as pH, methane or VFAs only change once inhibition is already advanced. Household inputs containing bleach, antibiotics and pharmaceuticals can disrupt anaerobic metabolism even at low concentrations, but current monitoring approaches cannot detect these effects early enough to prevent loss of performance. Volatile organic compounds (VOCs) offer a rapid, non-invasive readout of microbial activity and can shift within minutes to hours following stress.

We evaluated whether VOC fingerprints can reveal early metabolic responses to chemical stress using batch assays exposed to hypochlorite and sulfamethoxazole, complemented by a three-month monitoring campaign of septic tanks connected to a local community. VOCs were collected using SPME and thermal desorption and analysed by TD-GC-MS, with non-target data processed and diversity metrics.

Bleach caused an immediate 50% drop in methane production without affecting pH, highlighting functional inhibition despite stable routine parameters. VOC richness and Shannon diversity remained unchanged, but individual VOC features shifted significantly after chlorination, including transient compounds likely linked to oxidative stress. In the septic tanks, VOC community profiles showed substantial temporal variability driven by influent changes, yet specific VOCs consistently responded to chlorination and may serve as stress biomarkers.

This work provides proof of concept that non-target VOC profiling can detect metabolic disturbances before major performance losses occur. While community-level VOC patterns remained relatively stable, shifts in individual VOCs revealed chemical stress responses not captured by conventional monitoring. VOC analysis therefore offers a non-destructive, complementary tool for early warning in anaerobic systems. Future work will develop concentration–response relationships and identify metabolic pathways associated with stress-responsive VOCs to enable operational deployment.

7.02.P Advancing Pollution Control in Wastewater: Monitoring, Risks, and Regulatory Transitions

7.02.P-Th280 City-Scale Shotgun Metagenomic Surveillance of Antimicrobial Resistance across a sewage network that includes Urban, Hospital, and Airport Wastewater in Larnaca, Cyprus

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Wastewater surveillance is emerging as a key pillar of One Health monitoring of antimicrobial resistance. Building on studies from individual sites, this work provides a city-scale view by exploring how different human activity zones; urban, touristic, hospital, and airport, contribute to distinct microbiome and resistance profiles within the sewage network of Larnaca, Cyprus.

A two-year sampling campaign was conducted across ten geographical points within the sewage system: six urban pumping stations, the city General Hospital, the International Airport and the influent and effluent of the wastewater treatment plant (WWTP). This WWTP serves Larnaca's population equivalent of 72,000, a number that doubles during periods of high tourism. Composite 24-hour wastewater samples were collected every fourteen days. Shotgun metagenomic sequencing was carried out for diversity analyses, multivariate ordination, while a random-forest feature selection approach was used to characterize spatial and temporal patterns. Microbial source tracking was applied to evaluate the contribution of upstream locations to key sinks in the network.

Location was the dominant factor shaping microbial community, functional and antimicrobial resistance profiles. Hospital, airport and treated effluent samples exhibited distinct and reproducible signatures, while urban pumping stations showed similar characteristics, reflecting a shared urban input. Seasonal and temporal differences were minimal, possibly reflecting the minimal corresponding environmental condition differences. Urban pumping stations represented the primary contributors to the influent of the treatment plant due to their high wastewater flow. Despite representing a minor volumetric input, hospital wastewater accounted for ~15% of the resistome at the downstream urban pumping station that receives it, demonstrating its disproportionate impact.

This study shows that antimicrobial resistance patterns at the city scale are governed mainly by spatial structures and source-specific inputs rather than seasonal variation. The findings highlight the value of integrating metagenomics with source-tracking approaches to identify key contributors within urban wastewater systems and to support targeted surveillance and management under a One Health framework.

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7.02.P-Th281 Sampling Approaches for Wastewater-Based Epidemiology: Influences of Time-Weighted Composite and Grab Sampling through Time

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Wastewater-based epidemiology (WBE) has been crucial for tracking viruses and chemical usage and subsequently informing public health decisions. How and when to sample remains an important consideration because grab sampling during weekdays is common. We tested the hypothesis that viral RNA from four infectious diseases (using RT-qPCR) and chemical markers (pharmaceuticals using isotope dilution LC-MS/MS) would not vary between paired grab and composite sewage samples collected during Fall and Spring seasons in Texas, USA. We did not observe any significant ($p > 0.05$) differences for any viral targets between weekend and weekday sampling. However, SARS-CoV-2 concentrations in Spring were significantly higher ($p < 0.05$) than during the Fall season. We also observed significantly ($p < 0.05$) higher levels of N2 and C28311T in composite samples compared to grab samples.

Most chemical markers were significantly elevated ($p < 0.05$) during the Spring. Similarly, significant differences ($p < 0.05$) by sample type were observed for most compounds; most chemical markers were significantly

elevated ($p < 0.05$) in composite samples compared to grab samples. Atenolol, benzoylecgonine, sildenafil, and triamcinolone acetonide were significantly ($p < 0.05$) higher on the weekend, while concentrations of methylphenidate and amitriptyline were significantly ($p < 0.05$) higher in weekday samples. Though composite sampling is not consistently used in WBE, we observed significantly ($p > 0.05$) higher levels of virus targets and chemical markers in time-weighted composite samples.

7.02.P-Th282 Treatment of microplastics in wastewater treatment plants (WWTPs) case studies in Wallonia, Belgium: methods and results

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The occurrence of microplastics (MPs) in the environment has become a global environmental issue. According to a rough estimate 70-80% of marine plastics originate from land and are carried by rivers to the oceans. Currently, there is a rising concern about the role of wastewater treatment plants (WWTPs) in the release of MPs in the environment. Recent studies have shown that the presence of MP in wastewater is partly due to plastic microbeads contained in personal care products and plastic fibres released by the washing of synthetic fabrics. During treatment process in WWTP, around 99% of MP pass mainly into sludge.

In 2020, 69% of all WWTP sludge produced in Wallonia was recycled in agriculture. It is therefore important to evaluate the efficiency of Walloon WWTPs for the treatment of MPs. This study aims to assess the balance sheet. First, a sample preparation protocol has been developed to remove organic matter and separate MP by density difference. Then, a representative selection of WWTPs in Wallonia has been characterized in terms of MPs load in wastewater (inlet), in effluent (after treatment) and in sludge. Two analytical techniques were used to evaluate the number of MPs according to their size: Nile Red Microscopy for particles from 100 μm to 1 mm, and microFTIR spectroscopy from 20 μm to 200 μm . In addition, microFTIR spectroscopy gives the nature of MPs (PE, PP, PS...).

Next, a WWTP abatement rate was calculated from the data obtained. This is the percentage reduction in the number of MPs in the effluent (OUT) compared to the number of MPs observed in the raw water (IN). The abatement rate obtained between wastewater and effluent is 53-89%. This rate is close to that stated on publications on this subject. Regarding sludge, it is the receptacle for MPs, with a wide variety of particle shapes and discoloration observed. MicroFTIR data showed the presence of PET and PA in all three sample types. The sludge contains also PE, PP and PS. These are the most common MPs found in the environment.

7.02.P-Th283 Practical Experiences with Controlling Ozonation in Full-Scale Quaternary Treatment for Micropollutant Removal from Municipal Wastewater

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The revised Urban Wastewater Treatment Directive (2024/3019) requires advanced treatment for micropollutant removal at selected urban wastewater treatment plants (UWWTP's). In April 2025, the first full-scale quaternary treatment in Flanders was taken into operation at the Aartselaar UWWTP, combining ozonation and granular activated carbon (GAC). While literature recommends controlling ozonation via online dissolved organic carbon (DOC) or ΔUV measurements, practical implementation revealed several challenges.

Three CARBOVIS 705 sensors (WTW) were installed before ozonation, between ozonation and GAC, and after ozonation to measure UV absorption at 254 nm. These sensors allow measurement of other parameters such as dissolved organic carbon (DOC) based on site-specific calibration. The sensors feature ultrasonic and pressurized air cleaning, supplemented by weekly manual acid cleaning. Despite these measures, stable calibration of UV sensors against DOC proved difficult. Moreover, variability in laboratory DOC results across analytical methods makes it difficult to use this parameter. Use UV signal for operational control and insights will therefore be more robust than DOC-based interpretations.

Operational experience highlighted fouling as a major issue. The sensor positioned after ozonation was most prone to fouling, and weekly acid cleaning was insufficient to maintain stability. Rain events caused signal drift in all sensors hampering their use for process control. These findings underline the importance of sensor maintenance and the need for more advanced control strategies that take into account the limitations of the on-site sensors. While online UV monitoring offers a practical approach for ozonation control, its reliability depends on rigorous cleaning protocols and consideration of site-specific conditions.

This work provides insights to bridge the gap between theoretical recommendations and operational realities in full-scale wastewater ozonation systems. Lessons learned will inform best practices for implementing advanced treatment.

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7.02.P-Th284 Enhancing Pharmaceutical Removal via Photoelectrochemical Oxidation in Wastewater Effluent

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Organic micropollutants such as pharmaceuticals pose significant environmental and public health risks. These contaminants not only contribute to the spread of antibiotic resistance but also disrupt aquatic ecosystems. Advanced oxidation processes are known to effectively degrade pharmaceuticals, and among these, photoelectrocatalysis (PEC) offers a promising method for removing contaminants present at trace levels ($\mu\text{g/L}$ to ng/L). The focus of the presented study was to assess the influence of five quaternary ammonium compounds (QACs) on BiVO_4 photoanodes, modifying the structural properties and enhancing photoelectrocatalytic performance. The QAC-modified BiVO_4 photoanode variants were characterized using X-ray diffraction, scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-ray photoelectron spectroscopy, UV-Vis spectroscopy, and linear sweep voltammetry. PEC degradation experiments were conducted using effluent, collected after secondary treatment at a wastewater treatment plant, spiked with 10 selected pharmaceuticals at an initial concentration of $10 \mu\text{g/L}$. The removal efficacy of the modified BiVO_4 photoanodes was evaluated under simulated solar irradiation. Among the tested variants, the photoanode modified with the alkyl trimethyl ammonium compound ATMAC C18 showed the most rapid degradation, with several pharmaceuticals being removed within the first 15 minutes. However, dialkyl dimethyl ammonium compound DADMAC C18 modified photoanode was identified as the best-performing variant, with diclofenac degraded rapidly.

Keywords: Pharmaceuticals; Advanced Oxidation; Wastewater treatment; Photodegradation

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7.02.P-Th285 Exploring Biological Activity in Granular Activated Carbon during Full-Scale Quaternary Treatment at a Municipal Wastewater Treatment Plant

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The revised Urban Wastewater Treatment Directive (2024/3019) requires large wastewater treatment plants and those in sensitive areas to implement quaternary treatment for micropollutant removal. In April 2025, the first full-scale quaternary treatment system in Flanders was commissioned at the Aartselaar wastewater treatment plant. This advanced treatment combines ozonation and granular activated carbon (GAC), two complementary technologies that remove residual micropollutants and associated ecotoxicological effects.

It is theorized that the combination of ozone and GAC will result in a synergistic effect. Ozonation increases dissolved oxygen and converts residual non-biodegradable organic material into biodegradable organic matter in the water entering the GAC stage. Prolonged contact time between organic material adsorbed onto GAC, high oxygen content and biofilm growing on the carbon granules result in conditions that may promote adapted biofilms capable of degrading persistent dissolved organic carbon. Such biofilm activity could support self-regeneration of activated carbon, potentially extending its operational lifetime and improving micropollutant removal efficiency.

To explore this hypothesis, biological activity in the full-scale GAC is monitored through multiple approaches. Sampling ports allow collection of GAC from three levels of the carbon bed (top, middle, bottom). Oxygen concentrations are measured at inflow and outflow to assess redox conditions and oxygen uptake. Manganese uptake is monitored at the same points as an indicator of microbial activity, and manganese accumulation on the carbon bed is quantified to evaluate fixation as manganese oxide by aerobic biofilms. Micropollutant removal behavior is assessed at the full-scale GAC filters in an attempt to distinguish adsorption from potential biodegradation. Antwerp University characterizes biofilm activity through GAC ATP concentration and performs microbial community profiling via 16s RNA sequencing.

This research complements the bench scale research of Antwerp University within the same project and

provides new insights into the synergy between ozonation and GAC and the potential role of biofilms in enhancing micropollutant removal and operational sustainability in advanced wastewater treatment systems.

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7.02.P-Th286 Biodegradation of Imidacloprid in a Microbial Fuel Cell and Its Residual Toxicity to *Daphnia similis*

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The treatment of wastewater containing imidacloprid (IMI), a systemic insecticide, is challenging due to its lack of efficient removal through conventional processes. Several methods have been employed to treat IMI-containing wastewater, such as adsorption and advanced oxidation processes; however, tertiary treatment could enhance IMI removal in these wastewaters. In this work, was studied the biodegradation of commercial formulation of IMI (Galeão®) in a double-chamber microbial fuel cell (MFC), inoculated with the electroactive bacteria *Shewanella oneidensis* and *Clostridium butyricum*, respectively. The anode of the MFC received 2.3 mL of the freshly grown bacterial suspension (or sterile nutrient broth for the controls), 1 mL of insecticide solution and 96.7 mL of sterile distilled water, reaching a final working volume of 100 mL, while the cathode was filled with a 0.5 M potassium ferricyanide ($K_3[Fe(CN)_6]$) solution prepared in phosphate-buffered saline (PBS, pH 7.0). The analysis of possible intermediate metabolites was performed by gas chromatography coupled to mass spectrometry (GC-MS) and revealed the hydrolysis of the nitro group. The toxicological assay was conducted to determine whether the transformation of IMI in the MFC reduced its adverse effects on the organism *Daphnia similis*. The toxic effects were evaluated by comparing mortality and observable stress responses after exposure to untreated IMI solutions, which showed an LC50 of 19.84 mg/L, confirming the pronounced sensitivity of the species. For the samples treated in the MFC, a 1:10 dilution resulted in 100% mortality, while 1:100 dilutions produced approximately 85% mortality, and 1:1000 caused no mortality. These results place the EC50 between 1:100 and 1:1000 and indicate limited effectiveness of the treatment in reducing IMI toxicity. Similar patterns of high toxicity observed in controls without inoculum reinforce the conclusion that the MFC process did not significantly remove IMI nor reduce its harmful effects on aquatic organisms.

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7.02.P-Th287 Wastewater treatment plants in Wallonia (Belgium): their impact on endocrine disruption in aquatic environments and their treatment efficiency (STEP-PE project)

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Wastewater treatment plants (WWTPs) are recognized as sources of endocrine-disrupting (ED) compounds and micropollutants that may affect aquatic ecosystems. This study assessed whether discharges from 28 Walloon WWTPs influence chemical quality of receiving waters and fish health. Three complementary approaches were applied: (i) evaluation of ED activities with YES/YAS tests and (ii) chemical analyses of influent and effluent, and (iii) biological analyses of fish upstream and downstream of selected sites. Estrogenic activity, the predominant activity at both the entry and the exit, was markedly reduced after the epuration process, while anti-estrogenic activity increased. Androgenic activity was quantified in nearly 60% of influents but is almost absent in effluents, except at one site. Anti-androgenic activity appeared in effluents of seven WWTPs, often coinciding with loss of agonist activity. Overall, discharges from about one third of WWTPs impacted ED activity in the receiving waterbody downstream. Chemical analyses revealed phthalates as the prevalent contaminant family. Across all sites, the sum of concentrations for the 6 families studied decreased overall by 93%, except per- and polyfluoroalkyl substances (PFAS), which increased by 25%. Four substances—nonylphenol metabolite, DEHP, octocrylene, and DEET—exceeded 100 ng/L in effluents. Eleven compounds, including bisphenol A, several PFAS, and personal care products (PCP) such as triclosan and cashmeran, were

detected in more than half of effluents. Exploratory removal efficiency calculations indicated high elimination of chlorophenols, moderate removal of alkylphenols, phthalates, and PCP, while PFAS showed negative efficiency. Fish analyses revealed significant differences (increase) in estrogenic activity in bile downstream of one WWTP and in vitellogenin of male fish downstream of another, suggesting localized biological impacts. However, histological examination of male gonads showed minimal or no feminization effects across sites. In conclusion, WWTPs achieve substantial removal of many contaminant families, yet endocrine-disrupting activities and persistent substances such as PFAS remain of concern. These findings highlight the need for improved treatment strategies and monitoring to mitigate ecological risks and safeguard aquatic life in receiving waters.

7.02.P-Th288 Assessing Wastewater and Sewage Sludge Treatment Efficiency by Combining Physicochemical Characterization and in vivo Endocrine Disruption Bioassays

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Treated wastewater sludge contains nutrients valuable for agricultural soils but also carry contaminants, including endocrine-disrupting compounds. This study aims to assess the endocrine-disrupting activity of wastewater and sludge while evaluating their agronomic potential, addressing the need to balance environmental safety and nutrient recovery. Samples were analyzed using in vivo endocrine bioassays, phytotoxicity tests, and physicochemical analyses of nutrients, metals, and organic contaminants to provide an integrated assessment of risks and benefits. This abstract focuses on the results of endocrine bioassays, which capture complex biological responses to mixtures of contaminants found in sludge and wastewater.

Wastewater, sludge, and sludge liquor were sampled from a Parisian wastewater treatment plant (WWTP) at different stages of the treatment process during two seasonal campaigns, winter and summer, allowing evaluation of both solid and liquid phases, and seasonal variability. Samples were tested using two in vivo fish eleutheroembryo assays: the Rapid Estrogen ACTivity In Vivo (REACTIV) assay (OECD TG 252) and the Transgenic Eleutheroembryonic THYroid-Specific (TETHYS) assay.

A preliminary lethality test established the Maximum Tolerated Concentration for each sample. Results showed that wastewater toxicity markedly decreased after denitrification, indicating effective removal of toxic components. In contrast, sludge toxicity increased after the thickening stage that follows anaerobic digestion, suggesting enrichment of persistent compounds during dewatering.

Thyroid assays from the winter campaign revealed that thyroid activity in wastewater decreased throughout treatment and was no longer detectable in the effluent. However, variability was observed in thyroid activity of sludge samples between sampling weeks, possibly due to differing amounts of rainwater the WWTP has received. Estrogen activity was detected mainly in influent and raw sludge, implying that estrogenic compounds are effectively removed in later stages.

The results of the summer campaign are pending and expected by April 2026, providing insight into seasonal trends. Overall, while wastewater treatment effectively removes endocrine and toxic activity from the liquid phase, sludge fractions remain potential sources of endocrine-disrupting substances. Continued monitoring and optimization of sludge treatment are crucial to ensure safe agricultural reuse.

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7.02.P-Th290 Deployment and Performance Evaluation of a Biofilm-Based Passive Sampler for Urban Wastewater Monitoring

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The use of passive samplers (PS) for monitoring aquatic environments under the Water Framework Directive was recently made possible in France with the publication of the ministerial decree of April 26, 2022. However, the use of PS for monitoring wastewater is more complex due to matrices loaded with organic carbon, suspended solids, and debris. Specific PS adapted to this type of water have been developed and tested to address this issue. The Prebio cell, developed and commercialized by AdScientifique, is a PS based on in situ biofilm development, specially designed for deployments in wastewater. Within the program of the French

national laboratory for the monitoring of the aquatic environment (AQUAREF), the Prebio cell was tested during two deployment campaigns in the influent and effluent waters of a wastewater treatment plant in Oise region (France).

The first campaign aimed at verifying the applicability of the Prebio cell for wastewater monitoring and defining the optimal duration of deployment, with a view to issuing recommendations for their implementation. Twelve Prebio cells were deployed for 4 months in the influent and for 6 months in the effluent. They were retrieved at different times to follow the biofilm growth and the accumulation of alkylphenols and bisphenol A, analyzed by GC-MS. Grab samples were collected at the same time as the Prebio cells. Suspect screening was performed by a LC-QTOF to compare the range of the substances identified in the of biofilm samples and the grab water samples. The second campaign was conducted to compare the results of target chemical analyses of biofilm samples and water samples collected using automatic samplers averaged over 24 hours. Target analyses were performed by GC-MS and LC-MS, and focused on alkylphenols, bisphenol A, and per- and polyfluoroalkyl substances (PFAS).

The first campaign revealed that the Prebio cell was easy to use, as no power supply nor metrological checks were required. Optimal exposure times were defined for collecting sufficient biofilm and accumulating contaminants in sufficient quantities to conduct targeted analyses and suspect screening. During the second campaign, a comparison of the 24-hour averaged sampling method with the Prebio cell showed that biofilm can be used to determine the presence of the targeted contaminants in wastewater. The Prebio cell appears to be a promising tool that complements 24-hour average sampling for regulatory monitoring of wastewater.

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7.02.P-Th291 Lab-controlled evaluation of different passive samplers to monitor pulsed micropollutant levels

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Aquatic micropollutants often occur at incidental peaks, for example, during run-off from agricultural land after heavy rain, sewage overflow in urban areas, or periodic release of waste effluents from industrial processes. Such incidental peaks may be missed by occasional grab sampling, and the pollution concentration peak has often passed once effects are noticed. Therefore, field samplers that can integrate exposure over time and capture such pulsed events are needed. Ideally, the sampler facilitates extraction for chemical analysis and provides a time-weighted quantitative indication of the micropollutant levels. There are various types of samplers for aquatic systems in use, with different characteristics that determine their applicability.

Micropollutants have different chemical properties that influence the suitability of the applied sorbent material. In addition, micropollutants may be released from the sorbent into cleaner water in the period after a peak concentration. We evaluated POCIS discs, SPME fibers, and CLAM pump cartridges in aquaria spiked with a mixture of 10 pesticides at environmentally relevant concentrations (0.5 to 45 µg/L). Aquaria were either stirred or still, using 3 scenarios: 7 days of constant concentrations, a single 1-day pulse followed by 6 days of clean water, and a repeated 1-day pulse with 2-3 days of clean water before and after. Polyacrylate SPME fibers did not accumulate detectable levels of weakly hydrophobic pesticides, and rapidly lost most medium hydrophobic chemicals after each pulse. POCIS discs (HLB sorbent encased in teflon membranes) accumulated all pesticides, except the most hydrophobic one (afoxolaner, logP 6), retained them after the pulse, but were strongly influenced by water flow rate. CLAM cartridges (HLB) captured all tested chemicals, and although breakthrough to a second cartridge did occur, recovery rates (based on pump volume and spiked concentrations) were 50-130%.

7.02.P-Th292 Automated Passive Sampler Exposure Device (PASED) for Monitoring of Organic Contaminants in Water

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Anthropogenic organic contaminants in water are typically monitored using spot sampling, allowing the determination of concentrations at one point in time. Alternatively, time-weighted average (TWA) concentrations can be determined by passive samplers, such as Chemcatcher. However, temporal variation

within the deployment period cannot be assessed, making it difficult to identify pathways and point source events. Hence, an automated passive sampler exposure device (PASED) was developed, allowing the measurement of organic contaminants at increased time resolution (days instead of weeks) for the same operational costs by autonomously exposing passive sampler discs.

PASED was tested in the laboratory and in the field to ensure reproducibility, effective seal and linear uptake. Field tests were performed at a wastewater outlet pipe in the River Test, Southeast England. For the first 2-week deployment, one disc was kept sealed and three discs were exposed after one week. Chemcatcher cages were deployed alongside PASED, as well as up- and downstream of the outlet pipe. Samples were analysed using a targeted UHPLC-MS/MS method for 212 pharmaceuticals, plant protection products and their transformation products. TWA concentrations were calculated using three literature uptake rates.

In total, 121 (57%) contaminants were detected at least once during the first deployment at TWA concentrations from 0.00085 – 0.0014 ng/L (cilazapril) to 201 – 561 ng/L (fexofenadine). Hazards were generally low (HQ < 0.1 for 99% of observations), but high for diclofenac and imidacloprid at the outlet (HQ > 1). Only few contaminants (n = 11 to 37) were detected in the two field blanks and the sealed PASED disc at generally small concentrations < 0.1 ng/L. The deployment field blank and PASED blanked had fewer contaminant detections (n = 11 and 14) at lower concentrations than the retrieval field blank (n = 37), indicating that PASED seals effectively. There were no significant differences (p = 0.749) between the standard deviations of replicates deployed in PASED and Chemcatcher cages, suggesting a similar performance. However, TWA concentrations were lower in PASED, indicating a slower uptake, most likely driven by the higher flow rate in front of the Chemcatcher cage, 0.25 m/s compared to 0.11 m/s in front of PASED. Overall, initial results showed an effective seal, no blank contamination, and a similar performance to established Chemcatcher cages.

7.02.P-Th293 Integrated Ecotoxicological Approach with Advanced Chemical Analyses to Study Natural Toxic Mixtures from WWTP Affected River Waters

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Complex mixtures originating from wastewater treatment plants (WWTPs) often contain thousands of pollutants which enter the aquatic environment and may exert combined toxic effects on ecosystems and human populations. To address this complexity and to assess the overall hazard posed by environmental mixtures, an integrated approach combining chemical analyses with ecotoxicology is essential.

In this work we studied the toxic mixture from two WWTP-impacted river-waters by combining the exposure assessment, obtained from wide chemical target analysis of pharmaceuticals and trace metals, and the ecotoxicological assessment including both experimental methods and modelling approach.

The samples were collected from Lambro and Molgora rivers, both downstream from two WWTPs in the province of Monza-Brianza in Northern Italy.

Water sample has been extracted on SPE and the methanolic eluates were analysed by a high-resolution mass spectrometry based on Orbitrap principle. The overall toxicity of the samples was measured with the 72h algal growth inhibition test, the 48h *Daphnia* spp. immobilization test, the 96h zebrafish behavioral test. The algal and *Daphnia* tests were also conducted on SPE-organic extracts to calculate the median effective concentration (EC50) expressed as Relative Enrichment Factor (REF).

The exposure and the effect data were integrated following the iceberg modeling approach, which quantitatively compares toxic equivalent concentrations from bioassays (TEQbio) with chemical-analysis-derived toxic equivalent (TEQchem) to assess which amount of the observed toxic effects could be explained by the detected organic chemicals.

Preliminary results showed the presence of dozens of pharmaceuticals, while slight - moderate toxicity was measured with the three bioassays in the natural waters from both rivers. However, marked toxic effects on algae and on *Daphnia magna* were found in the adsorbable organic fraction with 72h-EC50 values of 8.8 and 13.3 REF in Lambro and Molgora samples, and 48h-IC50 of 31.06 REF in Molgora, respectively.

The integrated approach highlighted the main drivers of toxicities in both samples, however the comparison between TEQchem and TEQbio showed that the effects explained by the detected chemicals overestimate the observed one, suggesting the presence of antagonistic effects or chemical measurement uncertainties.

7.02.P-Th294 Integrated study of the water quality of the San Pedro River, Aguascalientes, Mexico: determination of physicochemical, toxicological and microbiological risk

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The San Pedro River in the state of Aguascalientes, Mexico, is the state's main surface water river. Over the last two decades, many pollutants have been dumped into the river, becoming a public health problem. Therefore, the objective of this research was to evaluate the physicochemical, toxicological, and microbiological characteristics of the state's main river. Eight collection sites were established along the river, and certain physicochemical parameters were determined for each sample. Three groups of aquatic organisms of different trophic levels (*Danio rerio*, *Daphnia pulex*, and *Raphidocelis subcapitata*) were used for toxicological analysis and were exposed to various fractions of the water samples (20, 40, 60, 80, 100%). Median lethal and inhibitory concentrations (LC50 and IC50) were determined, followed by acute toxicity units (aTU). Pathogen detection and typing were determined by endpoint polymerase chain reaction (PCR) and real-time PCR. The presence of resistance genes for the antibiotics oxytetracycline, florfenicol, and enrofloxacin was determined by multiplex real-time PCR. The physicochemical parameters showed dissolved oxygen values ranging from 2.17 to 5.8 mg/L, electrical conductivity values ranging from 508 to 3236 $\mu\text{S}/\text{cm}$, and total dissolved solids ranging from 358 to 1618 mg/L. Ammonium registered values greater than 0.5 mg/L in all sampling areas, nitrates ranged from 0.75 to 71.08 mg/L, nitrites from 0.30 to 73.60 mg/L, phosphates from 0.45 to 9.02 mg/L, and sulphates from 0.60 to 3.82 mg/L. The toxicological evaluation with *D. pulex* showed aTU values >2 in only two sampling areas; with *R. subcapitata*, five areas recorded aTU >2 , and with *D. rerio*, all collection areas showed aTU >2 . Among the pathogenic organisms identified in the water samples were *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Salmonella invadens*, and *Vibrio cholerae*. Molecular analysis revealed the presence of resistance genes to all three antibiotics in all sampled areas. Anthropogenic activities in the region have undoubtedly had a negative impact on river water quality, and even though water supply sources in Mexico have been recognized as a vital priority, the state of health of its water resources is discouraging.

7.02.P-Th295 Effects of metal-salt phosphate precipitation on riverine ion loads and biological endpoints: Insights from a two-year monitoring

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To meet increasingly strict regulatory thresholds for phosphorus, European wastewater treatment plants (WWTPs) are required to intensify chemical phosphate elimination. Metal salts are commonly used as precipitants, forming insoluble metal–phosphate complexes that are removed during treatment. However, accompanying anions (e.g., chloride, sulphate) and excess salts can enter receiving waters, increasing downstream salinity. Except for chloride, no binding environmental standards exist for salinity in Europe, so these inputs may go unnoticed, potentially altering chemical and ecological conditions.

To examine whether intensified phosphorus elimination affects downstream ion loads and biota depending on the type of precipitant, we conducted a two-year field study at a WWTP in Germany, during changes from aluminium-sulphate to iron(II)sulphate, and later to a mixed iron(II)chloride/polyaluminium-hydroxychloride formulation with variable dosing. Water samples were collected biweekly upstream, at the WWTP inlet and outlet, and at three downstream sites to capture spatial gradients. Standard cuvette tests were used to analyse nutrient loads, TOC, water hardness. Major and metal ions ($\text{Fe}^{2+}/\text{Fe}^{3+}$, $\text{Al}^{3+}/\text{Al}^{2+}$, SO_4^{2-} , PO_4^{3-}) were quantified using ICP-OES/MS and ion chromatography. To capture high-frequency dynamics, eleven conductivity loggers were installed along the river gradient and at the WWTP inlet and outlet, recording at 10-minute intervals. To assess ecological impacts, annual diatom and macroinvertebrate sampling was carried out before and after precipitant changes following standard protocols.

Preliminary conductivity data revealed pronounced seasonal variation, with lowest values in winter (224–492 $\mu\text{S cm}^{-1}$), despite occasional peaks associated with road-deicing, and highest values in summer (348–663 $\mu\text{S cm}^{-1}$), consistent with seasonal hydrology and temperature-dependent ion mobility. Maximum conductivities were consistently observed at the WWTP sites. Conductivity increased downstream of the WWTP but returned to upstream levels within 3–5 km, with a secondary peak 1.25–2.5 km downstream attributable to road runoff. Periods of aluminium-sulphate dosing were associated with elevated downstream salinity, whereas conductivity decreased following the precipitant change; upstream values remained stable, indicating that WWTP treatment was the driver. These preliminary trends will be further interpreted in conjunction with ongoing chemical and biological analyses.

7.02.P-Th296 Bridging Wastewater and Potable Water: Challenges and Recommendations

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Global freshwater scarcity for potable water production is intensifying due to population growth, deteriorating water quality, salinization, and rising demand. While arid regions are most affected, recent evidence shows that European countries with temperate climates also face increasing shortages, driven by extreme weather events and decreased river flow capacities. Despite ongoing efforts to enhance water use efficiency, alternative sources for drinking water are urgently required. Among unconventional options, direct and indirect potable wastewater reuse could offer a stable, cost-effective solution with established infrastructure and lower energy demands compared to desalination. Although EU policy promotes wastewater reuse, adoption for large-scale potable water reuse in the EU Member States remains limited, hindered by insufficient public awareness and a lack of supportive regulatory frameworks. Potable water production via wastewater reuse is particularly sensitive to the presence of pharmaceuticals, metabolites, and antimicrobial-resistant organisms—often referred to as contaminants of emerging concern—for which the health risks are unknown or no limit values have been established. Whether potable reuse is direct or indirect, it necessitates advanced treatment, comprehensive monitoring, sustainable management, public acceptance, and uniform governance. This presentation synthesizes the current state of the art and identifies knowledge gaps and key requirements for robust and safe potable water reuse. In addition to the research gaps identified from the literature, we consulted the opinions of international experts regarding knowledge gaps. Consequently, we propose strategic directions to enhance the reliable and sustainable use of reclaimed wastewater for drinking water production, thereby supporting EU climate adaptation and water security ambitions.

7.02.P-Th297 Prioritization of Relevant Micropollutants in Reclaimed Water use in Agriculture

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The reuse of reclaimed water for agricultural irrigation is increasingly promoted in water-scarce regions, particularly across the Mediterranean, but the presence of micropollutants in treated wastewater remains a concern for environmental and human health. Within this context, the NEWater project (Natural based and low Energy consumption unconventional solutions for the management of WATER sources) aims to develop energy- and cost-efficient hybrid biological, physical and nature-based treatment systems, validated across four large-scale demonstration sites representing wastewater effluents, agricultural drainage and household greywater. To harmonize monitoring efforts across partners, this study establishes a prioritized list of pharmaceutical compounds for reclaimed water irrigation scenarios, complemented by a specific list for greywater applications. An extensive literature review covering more than 60 studies and four participating countries identified over 150 pharmaceuticals and transformation products. A multi-criteria decision framework was applied, integrating occurrence, persistence, bioaccumulation and toxicity (OPBT), together with crop uptake potential, previous experimental evidence and alignment with EU policies, including Directive 2024/3019 and the EU Watch Lists. Catalonia was selected as a reference region due to its comprehensive dataset and pronounced water stress.

Based on the scoring criteria, eight pharmaceutical compounds were selected for the general list, including venlafaxine and diclofenac (scores of 6 and 5, respectively), followed by azithromycin, clindamycin, carbamazepine, fluoxetine, sertraline and O-desmethylenlafaxine (score 4). For greywater, where fewer studies and regulatory references exist, 10 additional compounds were prioritized. Triphenyl phosphate and benzophenone-3 ranked highest, followed by trimethoprim, bisphenol A, triclosan and caffeine. Due to limited information on greywater contamination, further substances with a total score of 4—such as 1H-benzotriazole, TCEP, acetaminophen and ibuprofen—were also included.

Overall, the general and greywater-specific lists provide a harmonized, adaptable framework that supports the evaluation of contaminant behavior in reclaimed water systems and their potential accumulation in soils and crops. The knowledge generated throughout the project will also enable refinement of the prioritized lists and guide future monitoring strategies.

Disclaimer/Disclosure:

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7.02.P-Th298 How to Find Persistent Chemicals in Discharges – Persistence-Directed Testing Combined With Target, Suspect and Nontarget Analysis

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Persistence is one of the most problematic properties of a contaminant entering aquatic environments. However, data on persistence from standardized simulation biodegradation tests is very scarce for chemicals in use. There are many pathways for chemicals to enter surface waters and one of the most significant is discharges from domestic and industrial wastewater treatment plants (WWTPs). Discharges from WWTPs are complex mixtures, containing non-monitored chemicals, unidentified transformation products, low-tonnage production chemicals, and UVCBs, that might include persistent compounds. Hence, the total number and type of persistent compounds in effluents is generally unknown and methods to assess these are needed. A novel persistence-directed testing approach is here presented where biodegradation simulation tests are conducted by diluting a whole effluent sample in an environmental water sample containing degrading microorganisms (biotic test systems). Test systems are incubated for 40 – 60 days and then analyzed by chromatographic methods. By comparing the peak area ratios between the biotic test system and the abiotic control, persistent features can be singled out. In this way, even unidentified chromatographic features (chemicals) occurring in an effluent can be categorized as persistent. Chemical analysis performed by high resolution mass spectrometry, allows a combination of target-, suspect-, and non-target screening. Environmentally highly relevant persistent features can then be prioritized during structure elucidation efforts. This approach has successfully been applied to evaluate persistence of chemicals in discharges from offshore oil platforms, where a much higher number and fraction of persistent chemicals were found by LC-HRMS compared to GC-MS analysis. Persistence-directed testing can now be applied to find persistent chemicals in WWTP discharges, which however requires method adaptation. The poster will address (1) the need for sample preparation and conservation prior to analysis, (2) the optimal combination of analytical methods for covering the chemical space of organic pollutants in WWTP discharges, (3) how the approach can inform regulation of persistent chemicals in case of high-confidence identification and (4) how the approach can enhance mechanistic understanding of structural characteristics that drive persistence. The poster will present the current state of development and invites for discussions.

7.02.P-Th299 The “rapidly biodegradable” criterion in the recast Urban Waste Water Treatment Directive: regulatory uncertainty and methodological implications for micropollutant exemption

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The recast Urban Waste Water Treatment Directive (UWWTD, 2024/3019) introduces an extended producer responsibility (EPR) for micropollutants based on polluter pay principle. In its current form only manufacturers of cosmetics and pharmaceutical products will be required to contribute to the financing of expenses related to the implementation of quaternary treatment to remove micropollutant. This EPR system shall be applicable by 31 dec 2028.

From an environmental standpoint, an exemption may be granted for certain micropollutants that are deemed rapidly biodegradable in wastewater or that do not generate micropollutants at end of life. Rapidly biodegradable in water is a new term in the regulatory world. However, for the moment, the directive provides neither an operational definition of “rapidly biodegradable” nor a list of accepted test methods, leaving a room for interpretation on the assessment of “rapidly biodegradable” criterion of micropollutant and preparing relevant information for exemption dossiers.

This work critically examines, among others, this criterion in the context of existing European Chemical legislation frameworks (REACH, CLP) and OECD guidelines for ready and inherent biodegradability. We analyze the extent to which these screening tests, conducted under standardized laboratory conditions, are transferable to urban wastewater systems, which are characterized by short hydraulic retention times, complex and variable biomass, fluctuating physico-chemical conditions and the presence of multi-component mixtures. A key question is whether “rapidly biodegradable” should be equated with ready biodegradability criteria, with maximum environmental half-lives (DT_{50} / DT_{90}) in matrices representative of sewer networks and treatment plants, or with an integrated weight-of-evidence approach combining experimental data, transformation product profiling and fate modelling.

We show that the absence of a precise definition and harmonized methodological framework increases the risk of heterogeneous data requirements for producers and inconsistent exemption decisions for micropollutants. Finally, we discuss options to operationalize the criterion (combinations of OECD tests, studies on activated sludge and real effluents, scenario-based modelling) and propose elements for scientifically robust criteria that could inform future implementing acts under the UWWTD and support a consistent application of the “rapidly biodegradable” concept across Europe.

7.03.P Advancing the Chemical Safety of Plastics: Bridging Science, Policy, and Innovation

7.03.P-Mo425 Enhancing the PlastChem Inventory for Chemical Prioritization in Plastics

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Plastics are a significant source of chemical exposure throughout their entire life cycle, posing risks to human health and the environment, and the achievement of a safe circular economy. The PlastChem inventory, launched in 2023, consolidated fragmented public data on 16,325 unique plastic chemicals, that is, substances that can potentially be present in plastics, and provided a framework for identifying and prioritizing chemicals of concern in plastics. We now present PlastChem v1.1, an upgraded version featuring a more robust database architecture with improved usability and reproducibility, refined curation processes, and expanded hazard and exposure information. Key updates include integration of the recent releases from FCCmigex, LitChemPlast, and ECHA food-contact material data, alongside rigorous CAS Registry Number verification. Hazard information has been refined using seven updated regulatory sources and offers greater granularity across harmonized classifications, self-classifications, and authoritative lists, including substances under evaluation in the EU. A major advancement is the incorporation of human exposure data from the FCChumon database, covering over 3,000 plastic chemicals detected in humans. In addition, backend refinements streamline the data consolidation, formalize manual curation steps, and enable the efficient extension and updating of the database, readying the platform for future dynamic integration with user interfaces. PlastChem v1.1 includes an overall higher number of unique chemicals in plastic, including those of concern. In addition, its improved identifier traceability makes it easier to prioritize plastic chemicals for regulation and prepare the database for future updates and expansions. The updated inventory presents an important support for global policy processes, such as the negotiations of the Global Plastic Treaty. It also offers a solid basis for monitoring plastic chemicals of concern, helping to design safer plastics, and assess environmental and human exposure. Periodic updates will keep the inventory relevant as chemical use and regulatory landscape evolve.

7.03.P-Mo426 Mapping the regulatory landscape of plastic additives across their lifecycle

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Background: A wide variety of additives are added to plastics during production to enhance the performance or appearance of plastic, rubber materials, and other polymers. As most additives are not covalently bound to the polymeric matrix, they can readily migrate from materials or micro- and nanoplastic particles (MNPs) during and after the often very short useful life of the product. Due to the ubiquity and multitude of uses of plastic materials, releases of plastic additives to their surrounding environment across their lifecycle are regulated across regulatory silos in multiple pieces of legislation with clauses related to chemical safety.

Aims: This study mapped common plastic additives, their function(s) in specific polymers to regulatory requirements for various uses of these chemicals and materials at different lifecycle stages. The aim was to identify and analyse commonalities and differences between regulatory processes for specific intended uses of plastics and/or associated additives.

Methods: Through a bottom-up approach, the 417 individual additives listed under ECHA's plastic additive initiative (PlasI), their function and the type of polymer in which they are typically used against the regulations listed in the ECHA's chemical legislation database (EUCLEF). EUCLEF is articulated around 90 lists of substances that typically correspond to annexes of 52 specific pieces of legislation. The text of all legislations was reviewed and was complemented by an analysis of relevant guidance documents, where appropriate.

Results and discussion: There are notable differences in the risk assessment and management of plastic additives across their lifecycle, and risk management can be driven by considerations other than toxicity (beyond hazard classification). The coverage of chemicals at different lifecycle stages also differs. A total of 181 PlasI substances are not regulated under any of the reviewed legislations, while 16 fall within the scope of most of these. Transport and environmental emissions apply to a small number of plastic additives. General product safety is currently opaque, whilst there are some notable differences in approaches between product-specific legislation. We highlight opportunities for improving the overall coherence and consistency of the regulation of plastic chemicals.

Disclaimer/Disclosure:

7.03.P-Mo427 Prioritizing plastic food contact chemicals and identifying structurally-similar chemicals

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The chemical safety of plastic food contact materials (FCMs) is critically important, due to the high likelihood for human exposure. Over 7'629 food contact chemicals (FCCs) are potentially used in, present in, or migrating from plastic FCMs, complicating hazard and risk management. To identify potentially hazardous plastic FCCs, we prioritized them using high-quality hazard and exposure data and identified structurally similar chemicals by grouping them. Overall, 807 plastic FCCs are known to be hazardous using GHS-aligned regulatory data. We ranked these hazardous plastic FCCs into four tiers: 88 are known to migrate from FCMs and are detected in human biomonitoring (Tier 1); 238 migrate but lack biomonitoring evidence (Tier 2); 161 are detected in FCMs but lack migration evidence (Tier 3); and 320 are listed for use but lack experimental evidence (Tier 4). However, at least 6'391 (84%) plastic FCCs lack hazard data, hindering hazard and risk assessment, and potentially leading to regrettable substitutions. Grouping structurally similar chemicals can help avoid replacing one hazardous substance with an equally hazardous, but untested chemical. To facilitate this, we developed a semi-automatic, modular, and quality-checked methodology for grouping chemicals into human-readable and structure-based groups, using SMARTS-pattern matching, curated list comparisons, and keyword searches. Of 7'629 known plastic food FCCs, 2'265 were assigned to priority groups with particularly many hazardous chemicals, including primary aromatic amines (38 known to be hazardous of 69 total), organotins (27 of 47), PFASs (26 of 94), metals (26 of 85), ortho-phthalates (20 of 63), hindered phenol derivatives (16 of 132), organophosphates (15 of 95), and siloxanes (6 of 44). Of 2'265 chemicals in priority groups, 345 are known to be hazardous, 235 pose other hazards (i.e., lower hazard categories or environmental), and 1'685 lack sufficient hazard data, highlighting the potential for regrettable substitution and key targets for future research. Note, not all known hazardous FCCs are assigned to a priority group. Our prioritization and grouping methods provide a practical, science-based, freely available, and scalable solution for better chemicals management in materials. It supports prioritization for regulation and targeted data gap filling, highlights chemical groups with the potential for regrettable substitution, and enables group-based regulatory approaches.

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7.03.P-Mo428 Suspect Screening of Plastic Chemicals in Plastics and Tissues from Arctic Marine Wildlife

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Plastic pollution introduces not only particulate debris but also thousands of plastic chemicals into the environment. Many of these compounds are potentially persistent, bioaccumulative, mobile, and toxic, yet their leaching from the plastic material and exposure to wildlife remains poorly understood. The Arctic, a sink for long-range pollutants, faces additional risks from plastic pollution, including plastic ingestion by seabirds and marine mammals and potential resulting chemical exposure.

In this study, we used gas chromatography- (GC-) and liquid chromatography- (LC-) coupled with a high-resolution mass spectrometer (HRMS) for suspect screening of plastic chemicals in Northern fulmars (*Fulmaris glacialis*) and ringed seals (*Pusa hispida*) from Greenland. Paired samples of bird muscle, liver, and plastic particles retrieved from their stomachs and liver samples from ringed seals were prepared using optimized extraction and cleanup protocols and analyzed on GC- and LC- HRMS. A suspect list of plastic chemicals was built upon the PlastChem database. A comprehensive suspect list and spectral libraries matching were applied on HRMS data. Statistical analyses included principal component analysis (PCA) to visualize chemical profiles and correlation analysis to assess chemical associations among paired samples.

We identified 79 and 55 chemicals via GC- and LC-HRMS, respectively. Among these 135 plastic chemicals, 111 were identified in ringed seal livers, 91 in fulmar livers, 94 in fulmar muscles and 101 in plastics recovered

from the fulmar stomachs. Plastics recovered from fulmar stomachs included three types: polyethylene, polypropylene, and polystyrene. The PCA revealed distinct profiles for the different samples analyzed with GC-HRMS, while LC-HRMS results indicated strong overlap of fulmar tissues and plastics, with ringed seal livers forming a separate cluster. Correlation analysis showed 42 plastic chemicals with positive associations across paired samples; 18 of them showed a strong correlation between plastics and tissue, suggesting plastic as the source of exposure to plastic chemicals. Of the 18 chemicals, eight are known to be present in or leachate from the three polymer types detected. Our findings demonstrate that Arctic wildlife species are exposed to plastic chemicals. The ingestion of plastic particles can be an exposure pathway; however, exposure from the food chain and the surrounding environment cannot currently be ruled out.

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7.03.P-Mo429 Quantifying Impacts of Packaging Choices in Consumer Baskets: Material Use, Environmental Impacts, and Chemical Releases

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The presence and release of hazardous chemicals and microplastics originating from food packaging has become an increasing concern for environmental and human health. While packaging policies, retailer initiatives, and consumer interest in sustainable options are growing, there remains a lack of integrated information linking packaging quantities, environmental impacts, circularity potentials, and chemical and microplastic release risks across real-world consumption patterns. This information gap limits the ability of policymakers, industry actors, and consumers to make informed decisions that reduce packaging-related burdens.

This study takes a first step toward closing this knowledge gap by combining detailed packaging data with representative food consumption patterns. We build on a comprehensive packaging database covering 3,750 retail food products and construct archetypical consumer shopping baskets, each reflecting distinct purchasing behaviors. For these baskets, we systematically quantify and compare packaging material types, packaging quantities, associated life cycle environmental impacts, material circularity potential, and the potential release of hazardous chemicals and microplastics throughout the use phase. This multidimensional assessment enables a comparison of packaging designs across products, consumer groups, and impact categories. By integrating multiple assessment dimensions, the study aims to advance a more holistic understanding of how packaging design choices and consumption patterns interact to shape environmental sustainability and human health outcomes. The approach is intended to enhance the evidence base for packaging-related policy development, support retailers in portfolio management and design decisions, and improve transparency for consumers seeking to reduce the environmental and health impacts associated with food packaging.

7.03.P-Mo430 Mapping Additive Flows Using a Functionality-Based Material Flow Analysis Model

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Plastics are used in a wide range industrial and consumer products, and their performance relies heavily on chemical additives. More than 10,000 chemicals have been identified to have been used in plastics, providing more than 20 functionalities (e.g. plasticity, flame retardancy, etc.). Due to their hazardous properties, the presence of plastic additives in primary and recycled materials raises concerns on the safety of plastic products and the quality of secondary materials. Despite these implications, there is no comprehensive overview of the mass flows of the major categories of plastic additives, including their levels in products. The objective of this study is therefore to explore a new way of mapping the flows of plastic additives over the entire lifecycle of plastics using dynamic probabilistic material flow analysis. Typical concentrations of additives for individual polymer-product category-functionality combinations were compiled from handbooks, product datasheets and other sources. These concentrations were analyzed to derive typical use patterns of additives by functionality (e.g., flame retardants) within individual product categories, which are then integrated into a material flow analysis model with a high resolution in the end-of-life flows to quantify the corresponding additive flows. Preliminary material flow analysis results show that higher amounts of additives are found in container and packaging flows due to high plastic consumption volumes and PVC flows due to the overall high additive content. Thus, this study reveals the general use patterns of different functionality categories of plastic additives and indicates the hotspots of additive flows during the use phase. Modelling additive flows can help to

understand the source of chemicals in products and waste flows, and assess the feasibility of mechanical recycling of individual waste flows in terms of the content and types of chemical additives therein.

7.03.P-Mo431 A Generic Approach for Estimating Environmental Release of Synthetic Polymer Microparticles (SPM)

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In 2023, the European Commission introduced a restriction on Synthetic Polymer Microparticles (SPM) under entry 78 of Annex XVII of the REACH Regulation, as laid down in Commission Regulation (EU) 2023/2055. This restriction prohibits placing on the market of polymers that meet the SPM definition—solid, synthetic, limited solubility in water, non-biodegradable, and <5mm (<15mm for fibres with a length to diameter ratio >3)—unless their use is specifically derogated. Derogations apply where releases can be prevented or minimized, or to avoid overregulation of certain uses and sectors. For derogated uses, suppliers must provide Instructions for Use and Disposal (IFUD) and report on estimated environmental releases of SPM to ECHA on a yearly basis (per legal entity) to monitor the effectiveness of IFUD and the restriction overall.

This poster presents a generic approach, E-calc_SPM, developed by the European Adhesive and Sealants Industry Association (FEICA), to support suppliers of SPM and SPM-containing mixtures in estimating environmental releases. The concept builds on emission estimation principles from Specific Environmental Release Categories (SPERCs), developed by downstream user associations for relevant lifecycle steps of chemical substances. E-calc_SPM applies conservative (Tier 1.5) assumptions to ensure broad applicability, which may lead to overestimations of environmental release. In addition, pathways to air, water, and soil are assessed using statistical evaluations (Eurostat data) of sewage treatment connectivity and sludge disposal practices across Europe.

It is acknowledged that due to the generic nature of the approach, there may be cases where a more detailed, site-specific or product-specific assessment increases accuracy. However, applying this concept enables companies to meet reporting obligations efficiently while maintaining flexibility for diverse operational contexts. It may also offer a practical solution for harmonizing environmental emission reporting of SPMs across organizations based on the widely accepted SPERCs.

7.03.P-Mo432 Regrettable Substitutions? Comparative Toxicology of Bisphenol Analogues in Marine and Mammalian Reproductive Systems

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Bisphenol A (BPA), a well-known plasticiser and endocrine disruptor, has been shown to have harmful effects and is restricted within the European Union. The 2024 regulation banning BPA in food contact materials has led to increased use of its analogues (e.g., BPS, BPAF). These substitutes may be “regrettable”, with toxicity levels similar to BPA both for human health and for the environment. To contribute to the development of New Approach Methodologies (NAMs) in reproductive toxicology, and having the One Health approach in mind, the toxicological potential of BPA, BPS, and BPAF was evaluated using different reproductive but related metrics: the development of embryos of marine invertebrates (e.g., sea urchin) and the viability and motility of bull sperm, using standardised or well developed protocols. Sea urchin *Paracentrotus lividus* embryo tests were performed after artificial fertilisation and development abnormalities emerged at concentrations of 0.049 mg/L for BPA and at 0.470 mg/L for BPS and BPAF, with the calculated EC50 values of 1.57 mg/L, 2.19 mg/L and 0.100 mg/L, respectively. At 5 mg/L (BPA) and 0.47 mg/L (BPAF), the development of embryos stopped at the morula stage. Bovine spermatozoa were treated with bisphenols (2–200 mg/L) for the analysis of cell viability (1h) and motility (0h and 1h). Viability was not affected by any of the compounds. BPAF significantly inhibited the progressive motility, whereas BPA and BPS produced no significant effect. The immobilisation of spermatozoa was significantly increased by BPA and BPAF (200 mg/L), whereas BPS produced no effect. Overall, the toxicity level followed the trend BPAF > BPA > BPS, with BPAF significantly affecting marine embryos and mammalian sperm motility, thus establishing a regrettable substitution for BPA. BPS showed relatively lower effect levels, although further studies are still needed. The results confirm the growing concern over the safety of the potential substitute BPAF and emphasise the importance of conducting additional mechanistic studies to assess the proper safety of new BPA substitutes.

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7.03.P-Mo433 Developing a Risk-Based Grading Framework for Recycled Plastics: Addressing Plastic Additives to Promote Recycling

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Promoting plastic recycling is an increasingly important issue from the perspectives of resource recycling and preventing plastic pollution. Determining the risk of the chemicals, such as plastic additives, contaminants, and by-products contained (hereinafter referred to as plastic chemicals) in recycled plastics is difficult because recycled plastics are made from a variety of plastic wastes, such as used plastic products and leftovers from the production of plastic products. However, unless such risks are assessed and managed, resource-efficient recycling of plastics will come to a standstill or remain limited.

This study aims to develop a grading concept based on a risk assessment framework for chemicals contained in plastics for waste plastics and recycled plastics, and to conduct a trial grading of recycled plastics.

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 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 degree of potential to be transferred from the media, which depends on the type of plastic resin or medium (air, liquid, or solid) that contacts the chemical in plastic; E is the exposure factor of the use scenario; and T is a parameter representing toxicity of the plastic chemicals, such as the upper exposure limit. The grade range is set based on parameters dependent on product use (M and E). We are currently gathering M and E to define grade ranges applicable to variable use cases and exposure scenarios for plastic products. Examples of the items which each grade will suit for are 1) food packaging, 2) skin care packaging, 3) daily goods such as stationery, and 4) item with minimal human contact such as flowerpots.

This study establishes risk-based grading framework for recycled plastics as a practical indicator. This enables businesses to apply appropriate recycled plastic use without specialized risk assessment knowledge and supports smooth distribution of recycled plastics.

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7.03.P-Mo434 Effect of Plastic Recycling on Polymer Characterisation

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This work focuses on studying the effect of the mechanical recycling processes on polymers. Polymer recycling can be classified into primary recycling (re-extrusion), mechanical recycling, chemical recycling and energy recovery. Mechanical recycling is a cost-effective and eco-friendly option for thermoplastic polymers.

Mechanical recycling involves several physical steps and is the most common polymer recycling method after energy recovery. This study applied mechanical recycling, comprising washing, drying, extrusion, and shredding to three recycled polymers: polypropylene (rPP), polyethylene terephthalate (rPET), and high-density polyethylene (rHDPE)). As all of these processes are physical, they can cause some structural and physical-chemical changes to the polymer. Three consecutive cycles were performed, and the initial and final samples (after three cycles) were analysed using FT-IR, SEM, and Py-GC-MS, to detect any structural and chemical changes.

FT-IR analysis revealed an increase in the crosslinking bands of the polymers, potentially due to the temperature

employed during the recycling process, resulting in an elevated polymer density. A decrease in the crystallinity bands was also observed. SEM analysis revealed smoother polymer surfaces with each recycling cycle. Few changes to the pyrolysis profiles were observed, and these changes did not affect the identification of the polymers or plastic additives. The effect of the mechanical recycling process was consistent across all three polymers studied.

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7.03.P-Mo435 Formulating And Recycling Of Conductive Polymer Composites For Sustainable 3D-Printed Printed Circuit Boards (PCBs)

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Electronic devices have been evolving towards better performances and new applications, but their production remains wasteful and ? is too great a challenge because of their small size and complexity. We focus our work on a versatile building block found in nearly all of them: Printed Circuit Board (PCB). Their architecture has evolved for reasons of functionality and safety into the layered copper/FR4 structure (fire-resistant epoxy resin reinforced with glass fibers) that is standard today. However, while this structure is well suited for versatility and high-volume manufacturing, it raises two major sustainability concerns. First, fabrication—particularly the etching step—requires an excess of copper and the use of solvents. Second, the thin, intertwined layers of different materials make efficient recycling difficult and lead to poor recovery yields.

To address these challenges, we investigated conductive materials compatible with additive manufacturing of PCBs by fused deposition modeling (FDM) as well as chemical recycling approaches such as selective dissolution and depolymerization, in order to delaminate and separate materials at the molecular scale.

We have formulated materials from different types of conductive charge (metallic, carbon-based and polymer) in a biobased plastics. While optimizing for good processability, namely extrusion and FDM, and checking for conductivity, we want additionally to develop a chemical recycling process through selective dissolution using Hansen Solubility Parameters.

Sustainability is improved both at the production phase —by using a non-wasteful additive method with less metal— and at the end-of-life phase.

7.03.P-Mo436 Towards a One Health Understanding of Artificial Materials: Research Infrastructure

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The increasing release of micro- and nanoscale particles from anthropogenic activities represents a significant and growing concern due to their unique physicochemical properties, environmental persistence, and potential impacts on ecosystems, food systems, and human health. To address this challenge, the FHERITALE project (Food, Health and Environment Research Infrastructures to Tackle Emerging Priorities, an EU-funded initiative) aims to establish an entry point for the European scientific community to investigate artificial materials at the micro- and nanoscale within a comprehensive One Health framework. The presented poster will present current project outcomes, including a broad survey conducted among European Research Infrastructures (RIs) to map existing services relevant to micro- and nanoparticle research across environmental, food, and health domains. Participating RIs provided detailed information on physical, chemical, in vitro, in vivo, and computational methodologies currently available for studying the behavior, uptake, and biological effects of artificial materials. The resulting service catalogue highlights the available analytical capabilities, including advanced microscopy, spectroscopy, mass spectrometry, NMR, and EPR techniques, molecular modeling, multi-omics platforms, and a

wide range of biological testing systems that span organoids, model ecosystems, and whole-organism assays. Early achievement includes mapping service availability across multiple RIs, as well as identifying technological gaps and outlining strategic priorities for strengthening interdisciplinary research capacity. The poster will also summarize other current FHERITALE achievements that integrate environmental, food safety, and human health research on artificial materials. In summary, the FHERITALE-related poster will promote cooperation across significant European risks, enabling harmonization of methodologies, increasing analytical capability, and enhancing the understanding of risks associated with micro- and nanoscale materials.

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7.04 Bridging the Data-to-Regulation Gap: How to Align NAMs with Regulatory Decision-Making

7.04.T-01 A Regulatory Window for Animal-Free Environmental Risk Assessment: Integrating New Approach Methodologies for Legacy Pharmaceuticals

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Upcoming revisions to the European pharmaceutical legislation present a timely window of opportunity to reduce vertebrate testing for legacy Active Pharmaceutical Ingredients (APIs). Under the proposed updates, the generation of environmental data will be required not only for new APIs but also for those approved prior to the introduction of the current environmental risk assessment framework. With over 1,200 legacy APIs lacking sufficient environmental data, compliance with these new requirements is likely to trigger extensive vertebrate testing. However, by integrating New Approach Methodologies (NAMs), such as in vitro and in silico techniques, into the revised legislation, there is significant potential to reduce reliance on animal use. Here, we propose a tiered decision-making framework that prioritises the replacement, reduction or refinement (3Rs) of animal testing through use of in vitro techniques and predictive computational models, with vertebrate in vivo testing for legacy pharmaceuticals reserved only when scientifically justified. This approach could prevent testing on more than 300,000 fish, reduce costs significantly, and support the EU's commitment to animal welfare. In light of the European Commission's ongoing development of a roadmap to phase out animal testing in chemical safety assessment, our proposal offers a practical opportunity to demonstrate the applicability of NAMs in regulatory decision making. It also provides a path towards the goal of vertebrate animal-free assessments without compromising environmental protection or patient access to medicines.

This presentation will outline a combination of factors which have aligned to provide an opportunity which, if taken, can enhance progress towards the understanding of how NAMs could be used in regulatory decision making. Critically, this opportunity on a selection of pharmaceuticals presents itself whilst also minimising the impact of the usual hurdles and blockers to the implementation of non-animal data

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7.04.T-02 AMiCl-BD: a QSAR Assessment Framework Compliant Machine Learning Model

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In silico approaches are relevant alternative methods to produce data for regulatory purposes. The release of the OECD principles for model and prediction validation forced the scientific community to develop and use only models considered as robust with a significant level of available information to meet authorities expectations. For KREATiS, the robustness of a model strongly relies on the quality of the data present in a model and this requires in-depth curation of the studies that cannot be simply automated. Following this philosophy, we developed a first model predicting the ready-biodegradability of organic chemicals with the aim to be acceptable for regulatory purposes. The AMiCl-BD (Aerobic Microbial Classification model for Ready-Biodegradability)

model is based on OECD 301/310 studies selected according to a stringent 3 step curation workflow resulting in a final dataset of 2183 substances split into a training, an internal validation and an external set. Finally, a random forest and a support vector machine model were developed according to the five OECD principles using optimized MACCS Keys fingerprints as model descriptors. First, the deep curation was a crucial step that required most of the time of model development and revealed numerous inconsistencies in experimental results. Second, the model exhibited very good goodness-of-fit, robustness and predictivity with a trend for conservative predictions. Importantly, this model demonstrates a well-balanced performance between precision and sensitivity (or specificity), particularly for compounds within its applicability domain. Third, the model provides as output a binary class along with a probability providing a level of confidence in the prediction. In addition, an automatic applicability domain assessment is implemented and a dynamic in-house reliability index is computed to support the trust of the users and authorities into the prediction. It is clear that machine learning approaches clearly suffer of a reputation of black box that may prevent their use for regulatory purposes. However, the AMICI-BD model tackles this drawback as possible by providing clear and accessible insight regarding the elements critical for the QSAR Assessment Framework.

7.04.T-03 Modernizing persistence and toxicity assessment through coupling biodegradation with an in vitro bioassay

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The conventional method of assessing the persistence (P), bioaccumulation (B), and toxicity (T) of chemicals is time-consuming, expensive and relies heavily on animal testing. This method cannot keep up with the drastic increase in global chemical production, resulting in a data gap in hazard profiles under the CLP and REACH regulations. To accelerate PBT assessment, novel hazard indicators, namely Cumulative Toxicity Equivalents (CTE) and Persistent Toxicity Equivalents (PTE), for the high-throughput screening of hazardous substances have been proposed. CTE represents the measured toxic effects of a substance using in vitro bioassays and PTE represents the toxic effects remaining after its simulated environmental degradation. Since the bioaccumulation reflects the internal concentration of a substance in relation to its toxic effect, bioaccumulation can be considered a part of toxicity assessment and therefore integrated into CTE and PTE. Six benzalkonium chloride surfactants (BACs) were selected to demonstrate the applicability of the CTE/PTE hazard indicators. CTE was determined using the MitoOxTox assay, which detects cytotoxicity, oxidative stress response and mitochondrial toxicity. MitoOxTox captures the three key events central to the adverse outcome pathways shared across major clusters of human disease but in the future a broader test battery will be applied. With increasing alkyl chain length, the cytotoxicity increased and effects in all endpoints were consistent with predicted baseline toxicity. We then applied a high bacterial density biodegradation test using sewage sludge, which had previously been demonstrated to mimic environmental degradation. BACs were nearly completely degraded (> 90%) within 72 hours. After biodegradation, the slurry was extracted by solvent extraction and the supernatant by solid-phase extraction. Subsequent bioassay testing revealed that effects decreased over time and correlated with the decrease of the BAC concentration. If transformation products (TPs) had been formed at all, their contribution to bioactivity was negligible. Therefore, we can conclude that BACs are not PBT chemicals, which is consistent with the results according to the PBT assessment in REACH (but now includes consideration of transformation products). This study demonstrates a workflow for fast screening of the toxicity and persistence but it remains to be expanded to more persistent substances and those that form problematic TPs.

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7.04.T-04 Practical Implementation of NAMs into Toxicological Assessment of Pesticide Residues

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The plant protection products may undergo various (a)biotic degradation processes that alter the original chemical structure of the pesticide after their application to crops and the subsequent entrance to the common food chain. As a result, a mixture of compounds can be formed, comprising the unchanged active substance (the parent compound), along with its metabolites and degradation products, all of them collectively known as pesticide residues. This variety of structures that could be formed, hurdles the toxicological assessment of pesticide residues by requiring significant efforts and resources.

The implementation of New Approach Methodologies (NAMs) offers new opportunities to improve the scientific and ethical basis of pesticide residues assessments. As highlighted by the European Food and Safety Authority (EFSA) and the European Chemical Agency (ECHA), the in silico approaches such as (Quantitative) Structure-Activity Relationship ((Q)SAR) modelling and read-across, can be practically applied to support the toxicological evaluation of structures, including metabolites, especially when experimental data are limited. In this respect, EFSA, in collaboration with ECHA, is currently running a project with the aim to develop a semi-automated workflow for bacterial gene mutation assessment of pesticide residues using the freely available QSAR Toolbox software and its interoperability with MetaPath (storing metabolism data) and IUCLID (storing toxicological data).

The workflow will consist of several key steps of analysis of the residues including: categorization based on their quantity, identification of major rat metabolites, gathering experimental data, searching for analogues for read-across purposes and (Q)SAR model predictions. Finally, the workflow will provide an overall conclusion on the bacterial mutagenicity for each structure. The user interaction is minimized to facilitate and streamline the work with the system and to ensure efficiency, reproducibility, and consistency in the assessment process. The logic implemented for each of the workflow steps aligns with the main principles of residue definition as recommended by EFSA and the forthcoming OECD guidance.

A pilot version of the semi-automated workflow has been already developed and tested on real case studies provided by EFSA pesticide applicants. Encouraging results are obtained, indicating the workflow as a practical tool to support the integration of NAMs in regulatory toxicology.

7.04.P Bridging the Data-to-Regulation Gap: How to Align NAMs with Regulatory Decision-Making

7.04.P-Tu444 Optimizing In vitro Battery for Genotoxicity Testing Using a Probabilistic Network To Support Decision Making

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Current strategies for genotoxicity testing use multiple in vitro and in vivo assays to evaluate three key endpoints: gene mutation, clastogenicity, and aneuploidy. Optimizing in vitro test batteries has become a priority for next-generation risk assessment (NGRA). Recent efforts introduced quantitative evaluation of the battery but may fail to adequately address uncertainties in assay accuracy and endpoint relationships. We developed a probabilistic Bayesian network that explicitly models such uncertainties and applies decision analysis to recommend stepwise testing protocols.

We constructed a probabilistic network based on the description of assay objectives in OECD guidelines. We adopted the data from Luijten et al. (2024) including 2,239 chemicals tested across ten assays. Due to missing values, we supplemented with TRAID project reporting assay sensitivity/specificity against rodent carcinogenicity. with 237 chemicals overlapping between datasets. Endpoint values, though unobserved, are essential for conditional probability estimation. We applied Bayesian latent class analysis, which enabled endpoint simulation from in vivo assay results. The conditional probabilities were estimated on the data of Luijten et al. (2024), TRAID and simulated endpoint values.

We evaluated the model's sensitivity to prior probabilities of carcinogenicity (0.1, 0.5, 0.9), reflecting potential in silico predictions in NGRA workflows. Genotoxicity was defined as positive if any endpoint was positive. Using the framework of Integrated Approach to Testing and Assessment, decision analysis assessed posterior probabilities under stepwise assay addition to identify minimal assay combinations for conclusive results and optimal sequential strategies.

The probabilistic network showed strong predictive performance, with 91% sensitivity and 85% specificity. Decision analysis revealed that a conclusive positive genotoxicity result required two positive outcomes from the HPRT and in vitro micronucleus (MN) assays. In contrast, a conclusive negative required three negative

results from HPRT, in vitro chromosomal aberration (CA), and in vitro MN assays. The bacterial reverse mutation (Ames) test provided limited information when combined with other in vitro assays.

Our probabilistic approach characterizes assay accuracy and relationships while providing a practical, stepwise testing protocol. We recommend initiating test batteries with HPRT and in vitro MN assays.

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7.04.P-Tu445 NAMs-based Assessment of Endocrine Disrupting Chemicals under K-BPR : Case Study on Preservatives

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As of 2025, a total of 368 preservative-type biocidal active substances—including product preservatives, film preservatives, and fiber/leather preservatives—have been notified as existing substances under the Korean Consumer Chemical Products and Biocides Safety Control Act (K-BPR). These notified substances face an approval deadline in December 2027, and preparations for their AS approval applications are currently underway.

Preservative-type substances are widely used across a broad range of consumer products, primarily to prevent product degradation and extend shelf life. Because these substances are designed to ensure long-term stability and preservation, they may also pose potential long-term hazards to human health and the environment. Additionally, concerns have been raised regarding the possibility of tolerance or resistance developing through repeated exposure. For these reasons, evaluating the potential endocrine-disrupting effects (EDCs) of preservative substances under chronic or repeated exposure conditions represents a critical hazard domain. Under the K-BPR, a substance is recognized as having endocrine-disrupting potential only when it is listed in the EU ED Lists I or II.

In this study, we aimed to screen preservative-type biocidal substances belonging to three product types that are currently undergoing the approval process under the K-BPR. Using TOXBAL, a DART model-based predictive tool, we identified substances with potential endocrine-disrupting activity, even if they are not included in EU ED Lists I or II. QAC benzyl-C12-16 (CAS 68424-85-1) and triclocarban (CAS 101-20-2) were selected for additional evaluation regarding their potential endocrine-disrupting effects. For these substances, we integrated NAMs-derived information with exposure pathways relevant to the Korean regulatory context and conducted a next-generation risk assessment case study to support regulatory decision-making related to endocrine disruption.

This study not only provides AOP-based foundational ED-related toxicological information for preservative substances undergoing approval, but also demonstrates the feasibility of conducting NGRA using NAMs. Such a framework is particularly useful for evaluating EDCs, where toxicological data are often limited, as it helps identify priority substances through mechanistic signals. For substances requiring further evaluation, the use of NAMs can support risk determination without relying on animal testing.

7.04.P-Tu446 Advancing Confidence in NAMs for Fish Acute Toxicity Assessment: Outcomes from the 2025 Innovate EcoSafety Summit

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The inaugural Innovate Ecosafety Summit brought together 57 experts from academia, government, industry, and non-profit sectors across 10 countries to accelerate the adoption of new approach methodologies (NAMs) in chemical safety assessment. The three-day event focused on two critical topics: replacing acute in vivo fish testing and applying NAMs in endocrine disruptor assessment for fish and amphibians.

The acute fish toxicity discussion session provided an opportunity for breakout groups to evaluate example datasets under three regulatory contexts: Deriving a Predicted No Effect Concentration (PNEC), deriving a Fish-

Specific Risk Quotient (RQ), and Classification & Labelling (CLP). Confidence in NAMs was highest when data were corroborated by QSARs and the mechanisms of action were well understood. Conversely, confidence dropped when evaluating reactive compounds, scenarios where there were dataset discrepancies, or where there was a lack exposure data (relevant to the risk-based contexts).

Discussions indicated that confidence in using NAMs is shaped not only by data quality but also by the clarity of frameworks and through collaborative interpretation. Participants emphasized the need for consistent terminology, mechanistic understanding, and better integration with exposure models. The use of data-driven case studies highlighted where NAMs add value, and where gaps remain.

Next steps include developing further case studies and establishing criteria for data applicability in specific contexts. The Summit underscored the importance of cross-sector collaboration and regulatory evolution to reduce vertebrate testing while maintaining robust environmental protection.

7.04.P-Tu447 Towards NAM-based Frameworks for Assessing Endocrine Activity in Aquatic Vertebrates: Outcomes from the 2025 Innovate EcoSafety Summit

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The 2025 Innovate EcoSafety Summit convened a global, cross-sector community to advance predictive, non-animal approaches for chemical safety assessment. A central focus was the development of prototype frameworks for evaluating endocrine activity (specifically for the estrogenic, androgenic, thyroid, and steroidogenic (EATS) modalities) in aquatic vertebrates without reliance on traditional in vivo assays. Breakout group discussions, balanced across academia, industry, and government organisations, explored the essential components of such frameworks under hazard- and risk-based decision-making paradigms.

The discussions revealed strong convergence on a tiered, mechanistic framework that begins with existing data and escalates when uncertainty persists. Core elements included integration of in silico, in vitro, and cross-species endocrine activity data, with an emphasis on the need to demonstrate transparency, reproducibility, and weight-of-evidence approaches. Risk-based groups highlighted the critical role of exposure data and in vitro to in vivo extrapolation tools in reducing uncertainty, while hazard-based groups emphasised precautionary principles (i.e. the need for generation of higher-tier hazard data to address uncertainty), and the use of qualitative thresholds.

Key outcomes included the need for shared terminology, context-specific confidence criteria, and structured validation of novel approaches. Next steps identified include mapping assay coverage for thyroid and steroidogenesis pathways, piloting integration tools, and developing user-friendly guidance for regulators and data submitters.

This session lays the groundwork for a follow-up activity in 2026 to stress-test such prototype frameworks, which will have an initial focus on estrogenic activity, using real-world data. This activity will identify what further work is needed to ensure sufficient confidence to enable the regulatory acceptance of NAMs and reduce reliance on in vivo testing in aquatic vertebrate endocrine disruptor assessment.

7.04.P-Tu448 Establishing Molecular Size and Log P as Reliable Indicators for Worldwide Bioaccumulation Assessment: A New Decision Tree Screening Approach

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Since the implementation of REACH, various parameters have been used to screen and prioritize chemicals, with log Kow as the primary criterion for bioaccumulation. ECHA guidance also recommends parameters such as maximum molecular diameter to support conclusions on a substance's lack of bioaccumulative potential. To our knowledge, these criteria have not been systematically re-evaluated using the wealth of new data now available.

In this study, we compiled a dataset of 655 compounds with publicly available experimental fish BCF values. For each compound, 4,179 molecular descriptors were calculated using AlvaDesc. Feature selection employed sparse Partial Least Squares (sPLS) to identify the 20 descriptors most strongly correlated with bioaccumulation

(log BCF ? 3.3). Decision trees were trained to generate transparent classification rules using empirical class priors and an asymmetric cost matrix heavily penalizing false negatives. The probability threshold (τ) was optimized via repeated, stratified five-fold cross-validation (ten repetitions), first minimizing false negatives, then reducing false positives. Rule stability was assessed through extensive bootstrapping, retaining only trees with zero false negatives and aggregating recurrent rules and split variables.

Our results indicate that an ultra-conservative threshold ($\tau ? 0$) is required to achieve zero false negatives, which increases false positives at the screening stage. The most recurrent decision rules combine hydrophobicity, molecular size, and topological descriptors (log Kow, Size3, BLTD48), refined by polarity (e.g.TPSA.Tot.). These patterns are chemically plausible and align with bioaccumulation mechanisms and main regulatory cut-off values. The main discriminating descriptor was log Kow (threshold ?4.5), followed by Size3 derived from average molecular diameter, consistent with ECHA Guidance R.11.

This data-driven analysis confirms that the log Kow and size thresholds currently applied under REACH are appropriate, robust, and enable a highly protective approach (no false negatives). We propose a decision-tree workflow integrating multiple descriptors into a science-driven, transparent, and highly sensitive tool, ensuring protection through a conservative, zero-false-negative strategy.

7.04.P-Tu449 Science without borders: State of the art of the regulations on the use of NAMs under EU REACH and K REACH

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New Approach Methodologies are defined as “any technology, methodology, approach, or combination that can provide information on chemical hazard and risk assessment to avoid the use of animal testing”. The European Commission is set to publish a roadmap in early 2026 to phase out animal testing in chemical safety assessments, accelerating research initiatives and the development of NAMs as alternatives to animal testing. However, regulatory practices and acceptance of NAMs tend to evolve at a slower pace. Indeed, there are several hurdles to the integration of NAMs in a regulatory framework: ranging from the need to gain confidence (via standardization and formal validation by the OECD), to the current regulatory framework that cannot yet accommodate this new type of data. In Korea, the regulatory landscape shows a similar yet distinct trajectory. Under the Act on the Registration and Evaluation of Chemical Substances (K-REACH), a major amendment in 2018 mandated the prioritization of vertebrate animal alternative testing and prohibited duplicate animal tests for the same chemical, establishing a clear legal basis for encouraging NAMs. Korea has also strengthened institutional capacity through KoCVAM, which participates in international validation efforts and supports the development and evaluation of alternative test methods. While these legal and organizational steps signal strong policy intent, the practical regulatory uptake of NAMs remains gradual. In this review, we sought to establish a comparison of the regulation on the use of NAMs under REACH regulation in Europe vs Korea. We conducted a comprehensive analysis ranging from the legal basis for the use of NAMs to policy roadmaps and several concrete case examples. In addition, we investigated whether our own NAM portfolio matched the criteria for regulatory acceptance and how it could be improved for a better fit for purpose approach for NGRA. This study aims to provide insights for researchers wishing to develop NAMs fit for regulatory purposes, and to strengthen international harmonization of regulatory practices and favor the use of existing data across borders to reduce animal testing.

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7.04.P-Tu450 In-Depth Comparison of Predictivity Performances of Regulatory Compliant Ready-Biodegradability Models

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Ready biodegradability is a key endpoint for the environmental hazard assessment of chemicals in the framework of different regulations. Many freeware models are known to predict the ready biodegradability of

organic chemicals and several of them are regularly used for the completion of regulatory dossiers like Biowin, VEGA SARpy and OPERA R-Biodeg models. Recently, KREATiS released a new model following as possible its philosophy of model development: a deep and manual curation of the dataset to reject as possible the non-reliable studies, a mechanistic understanding of the predicted endpoint and total compliance with the QSAR Assessment Framework set by the OECD in 2023. This new model, called Aerobic Microbial Classification of Ready-Biodegradability (AMiCl-BD), contains two modules: a Random Forest and a Support Vector Machine (SVM)-based ones. The aim of this poster is to present predictivity performances of these five models tested along a same dataset, discuss the impact of the applicability domain as defined by the model developers and discuss these results in the light of the characteristics of the models (e.g. training set, descriptors, model choice). To be fair, the applicability domain of each model was defined automatically with no in-depth check of the data (e.g. analogues selected for applicability domain indices for AMiCl, VEGA, OPERA) meaning that the statistical values can be considered as worst-case. This analysis is based on 1135 substances that are not present in any of the training set of these models. The predictivity statistics were computed two times for each model: for all the predicted compounds and only for those falling inside model applicability domains, as detailed by the model developers. A first observation is that strong differences of accuracy and precision were observed between the models before and after the applicability domain assessment. Applying a strict applicability domain enhanced the performance of most models. In other words, these models predict less but with more confidence. If VEGA displayed satisfying statistics for the negative predictivity, the AMiCl ones presented the best balance while predicting ca. 1.6 times more substances falling inside applicability domain. Also, the fact to set a strict applicability domain by default led to the rejection of numerous predictions correctly predicted with AMiCl, especially for the non-readily biodegradable compounds.

7.04.P-Tu451 Innovate EcoSafety Summit: A Collaborative Model for Advancing Environmental Risk Assessment

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The Innovate EcoSafety Summit was established to provide a dedicated forum for cross-sector discussions on advancing environmental risk assessment. The inaugural meeting focused on the use of new approach methodologies (NAMs) for estimating acute fish toxicity and screening for endocrine activity in fish and amphibians. Although method development is progressing in both areas, regulatory use has been limited by a lack of clarity on expectations for method performance, confidence in the methods, and guidance on interpretation.

To address these challenges, the summit combined plenary presentations with breakout discussions. Mixed-sector groups were tasked with evaluating case studies under defined scenarios and identifying where NAM data were sufficient and where key gaps remained. For acute fish toxicity, groups evaluated datasets inspired by real chemicals across different regulatory contexts (e.g., classification and labeling, derivation of predicted no-effect concentration (PNEC), and fish-specific risk quotient calculation). For endocrine activity, groups were asked to outline components of a framework for assessing estrogenic, androgenic, thyroidal, and steroidogenic activity using NAMs.

For acute fish toxicity, participants identified clear conditions under which NAMs can be used with confidence (for example, when the mode of action is well-characterized and within the known applicability domain of the methods, there is concordance across different lines of evidence, and decision contexts are less sensitive to quantitative uncertainty). Factors that reduced confidence included metabolic uncertainty and discordant datasets. Discussion on endocrine activity revealed general agreement on the need for a tiered testing strategy, but also a varying level of confidence in negative results. Across both themes, the discussions emphasized the need for clearer terminology, defined applicability domains, and increased familiarity with NAM datasets. Participants identified short-term actions to advance implementation, including training through scientific and regulatory forums, developing case studies, clarifying decision criteria, and providing guidance to support consistent submission and evaluation of NAM data. These discussions underscored that continued progress depends on shared interpretation, coordinated development, and cross-sector collaboration.

7.04.P-Tu452 Responding to Regulatory Concerns on Air-Breather Bioaccumulation with NAMs: Extending Existing In vitro Rat Liver Assays - Developing and Validating a New Test Guideline

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In June 2017 ECHA revised its Chapter R11: PBT/vPvB Assessment document to include new screening threshold criteria for bioaccumulation in air-breathing organisms linked to Kow and Koa (log Kow >2 in conjunction with log Koa >5). For substances screening as potentially bioaccumulative in air-breathing organisms, generation of further information, such as toxicokinetic (TK) data (e.g. OECD 417), may be necessary, which would be both animal and resource intensive. A significant percentage of REACH registered substances fulfill the proposed cut-off thresholds, and would thus, necessitate further assessment. No test guidelines nor tiered framework for generation of lower-tier screening data exists. This inspired the 4-year CEFIC LRi programme of ECO 41, Enhanced Screening Methods to Determine Bioaccumulation Potential of Chemicals in Air-Breathing Species, which consisted of 3 work packages: 1) Propose and evaluate a screening test to establish Koa; 2) Review and compilation of existing data on bioaccumulation and TK for air-breathing species; 3) Develop an In vitro NAM (new approach methodology) for assessing bioaccumulation. The In vitro Rat liver S9 assay developed offers a Tier 2 NAM approach and can be used to de-prioritise chemicals from eventual Tier 3 In vivo studies. The predictive capacity of this testing protocol was demonstrated to be fit-for-purpose and is ready for validation.

Several other national and international initiatives have followed-on from this, including: i) the European Chemicals Agency (ECHA) work group on Toxicokinetics and Bioaccumulation Assessment of Air-Breathing Mammals, who reported their findings in November 2022; ii) the Umweltbundesamt project Integrated Strategy for the Assessment of Aquatic and Terrestrial Bioaccumulation which considered in vitro rat hepatocyte and liver S9 data, and iii) the US-EPA led SPSF (Standard Project Submission Form) to develop an OECD Test Guideline for determining human intrinsic clearance rates and protein binding capacities, methods that are closely related to the rat depletion assay.

This presentation will review and assimilate the work performed through the various projects in the domain of air-breather B, consider commonalities and differences between findings, and, propose a pathway forward to consolidate the existing information, identify remaining gaps while suggesting solutions to elevate suitable NAMs to a status of regulatory acceptability to fill the air-breather B end-point.

7.04.P-Tu453 AI in chemical regulation: Current approaches and future potential for integrating AI within chemical hazard and risk assessments

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Chemical regulation procedures are becoming increasingly complex within the European Union (EU) coinciding with the continued drive towards reduction of animal testing (e.g. New Approach Methodologies NAMs). Thus, there is an urgent need to make the most of existing information and alternative (new) methodologies.

Meanwhile, machine learning (ML) and artificial intelligence (AI) have been rapidly developing and could provide very powerful tools in addressing these opposing regulatory requirements.

The European Food Safety Authority (EFSA) has recently published an outline of their vision of the use of AI for regulatory risk assessment for pesticides. However, no harmonized regulatory AI tools or official guidance for their use in risk assessment, are available. Consequently, the use of these tools is often limited to tasks such as supporting with literature searches and reviews, summarising large regulatory documents and writing code for computer models and big data.

AI has the potential to be beneficial in the development of data-intensive and expert driven NAMs (e.g. in vitro assays, in silico models, omics technologies) by making them more accessible and democratic for regulators and other stakeholders. For example, AI could assist a regulator with reviewing the validity of code for a novel model submitted by an applicant. This improved potential to develop NAMs, while concurrently ensuring the ability of regulators to review these methods, could be of particular use in avoiding animal testing for low-toxicity substances which may only require a screening assessment for toxicity.

While advanced ML tools offer these promising capabilities, they often lack transparency required to be accepted as standalone decision-making tools. Key limitations and concerns with current ML approaches are hallucinations and bias of generative AI; environmental impacts of a large scale deployment; and the link of cutting-edge technologies with extant regulations. Therefore, although AI should have a significant impact on the in bridging the gap between NAMs and regulatory decision making, the development of AI tools and guidance will need to involve stakeholders from all parties (regulators, academia and industry) for its success. This poster will discuss how AI is currently being used in a regulatory environment (by risk assessors as well as regulators); what are the major concerns and the current limitations of these technologies how such tools may develop in the future.

7.04.P-Tu454 Are We Under the Illusion of Control? Looking Beyond Quantification to Uncover “Real” Environmental Concentration Distributions of Chemicals

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Understanding data limitations is vital in an effort to benchmark the reality of exposure models against monitoring data and transition to a systems-based, more effective, and resource-efficient regulatory environmental risk assessment. The “real” distribution of pesticide concentrations in surface waters is only partly represented by monitoring. Measured environmental concentrations below their limit of quantification offer partial info that can help reconstruct the “real” environmental concentrations.

This study compared multiple statistical approaches to: (1) determine appropriate methodology for handling censored MEC distributions, (2) apply optimal methodology combinations to approximate REC distributions, (3) contextualize RECs with substance-specific and environmental parameters, and (4) analyze environmental risks for aquatic non-target organisms based on assumed REC-derived exposure.

Monitoring data (n=39 mil.) from 43,202 sites across 34 countries (2001–2023) were collected, containing 1,958 substances (11% pesticides). After curation and outlier removal, we developed a tiered hybrid modelling strategy: MEC normalization with median LOQs combined with multiple-censored maximum likelihood estimation for moderate censoring (<70%), Helsel's regression on order statistics for high censoring (70–90%) or multiple detection limits, and quantile random forest as fallback.

Censoring proportions ranged 1–100% (median=36%) across substance classes. Sampling frequencies varied substantially between organic (median monthly=1), pesticidal (2), and inorganic chemicals (2.5); maximum frequencies vary from organic 4–5, inorganic 5–18, pesticides 6.5–250 samples monthly. Organic non-pesticides are quantified more frequently than organic pesticides. Spatial analysis revealed higher concentrations for inorganic chemicals across Europe; pesticides exhibited higher median concentrations than non-pesticide organics but lowest detection rates above LOQ.

By combining measurement normalization with tiered hybrid modelling, this study addresses a critical methodological gap in handling censored water quality data at a continental scale. Emerging spatial-temporal patterns, particularly pronounced pesticide contamination relative to organic non-pesticides, provide foundation for threshold integration and risk prioritization. Ongoing work includes risk characterization, model validation, and integration of supplementary explanatory data (e.g., pesticide application rates).

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7.05.P Enhancing Chemical Safety using NGRA: Bridging Human Toxicology and Ecotoxicology

7.05.P-We432 Biomarker Signatures for Detecting Chemical-Induced Developmental Delays in Zebrafish Embryo Model

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Chemicals can disrupt normal embryonic development, resulting in embryo toxicity, developmental delay or abnormal development. Detecting developmental toxicity is critical for both humans and the environment. Zebrafish embryos are widely used in developmental toxicology, supported by advances in automated image-based assessment of structural features. To improve chemical comparison and link effects to mechanisms of toxicity, identifying biomarkers for developmental delay is crucial. To establish morphological markers for developmental delay in zebrafish embryos at the 96 hours post fertilisation (hpf) reference stage, we quantitatively characterised the morphological progression from 72 to 96 hpf using the Automated Imaging Robot (AIR). Features were quantified using FishInspector software and analysed with KNIME workflows and R scripts. For validation, embryos were incubated at different temperatures (24.5–27.7°C) and compared with structural features at 96 hpf. Using regression analysis, structural features with stage and temperature dependency were identified. An existing dataset of ~200 chemicals (PrecisionTox project) with quantitative morphological effect data was used to identify compounds primarily causing developmental delay. The stage

and temperature dependency of 48 structural features were evaluated with heatmaps and principal component analysis. Seventeen of these features strongly correlated ($r^2 > 0.8$) with developmental progression. Of these, nine also showed strong temperature dependency and were selected as robust biomarkers of delayed development. Using the PrecisionTox chemicals, 56 chemicals showed effects on one or more of the nine biomarkers. One compound, 4-methylimidazole, had effects on all nine biomarkers and showed greater sensitivity for these biomarkers (median EC50=6,206 μ M) than for other morphological features (median EC50=12,849 μ M), indicating that developmental delay was the primary effect. At higher concentrations, other structural features unrelated to developmental delay, like notochord curvature were identified. Our study established and validated nine developmental delay biomarkers in zebrafish. These biomarkers provide mechanistic links to modes of action, distinguishing compounds that primarily slow development from those causing other developmental effects. They can aid in prioritising chemicals with potential developmental toxicity relevant to environmental and human health.

7.05.P-We433 Harmonizing NAMs Across Species: Molecular Docking and *Caenorhabditis elegans* for Endocrine Disruptor Screening

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The increasing global production of plastics has raised concerns about additive chemicals—including plasticizers, stabilizers, antioxidants, and other functional agents—that can leach from polymer matrices and lead to widespread human and environmental exposure. While regulatory attention to endocrine-disrupting chemicals (EDCs) and their association with plastic additives has increased in recent years, hazard information for numerous plastic additives remains limited.

Here, we applied an integrated new approach methodologies (NAMs)-based framework combining in silico molecular docking and in vivo *Caenorhabditis elegans* assays to efficiently screen the endocrine-disrupting potential of plastic additives. Molecular docking of 417 EU REACH-registered additives was performed against human estrogen and androgen receptors (ER α and AR), followed by cross-species docking using AlphaFold-predicted structures of the *C. elegans* nuclear receptors NHR-14 and NHR-69, which are putative orthologs of human ER α and AR, respectively. Chemicals showing strong binding interactions across species were further examined through a systematic literature review focused on ER- and AR-mediated effects. By integrating evidence, 2-(2H-benzotriazol-2-yl)-4-methylphenol (UV-P) and 1,3-dimethyl-2-benzylimidazolium bromide (DBM) were identified as potential ER and AR disruptors and were subsequently validated through reproductive toxicity assays using *C. elegans* wild-type N2 and loss-of-function mutant strains (nhr-14 and nhr-69). This NAMs-based framework directly aligns with Next Generation Risk Assessment (NGRA) principles by linking molecular initiating events (MIEs) to adverse outcomes (AOs) through mechanistic understanding. By connecting in silico predictions (Level 1) with non-vertebrate whole-organism models (Level 3-4) within the OECD EDC assessment framework, this approach demonstrates how cross-species MIE conservation assessment can expand the utility and predictive power of alternative animal models while adhering to the 3Rs principles. The cross-species docking strategy exemplifies how NAMs can harmonize mechanistic data across species, supporting unified chemical risk assessment aligned with the One Health framework.

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7.05.P-We434 Estimation of Intra-Specific Ranges of Genetic Susceptibility to Toxicity Using Genetically Diverse Populations of Human Lymphoblastoid Cell Lines

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Aim of the study is to provide a greater understanding of the genetic sources of variation that underlie individual susceptibility to toxicity. Consequently, we have carried out high throughput screening of thirteen chemicals (Cadmium chloride, Sodium (meta)arsenite, Acrylamide, 4-Methyl imidazole, Dexamethasone, Cyclosporin A, Tamoxifen, Genistein, Rotenone, Tetraethylthiuram disulfide, Valproic acid, 5-fluorouracil and DMSO) in 261

lymphoblastoid male and female cell lines from 18 populations to discover genetic variability and sex-dependent changes in toxic responses in humans.

Clear response differences to all toxicants were found and ANOVA analysis of the cell line dose-response toxicity data from the populations revealed significant intra-population sex differences and population specific responses to the toxicants studied.

Further aim is to evaluate the degree of certainty in causal links between molecular key events and chemical risks to human health. Thus, the role of single nucleotide polymorphisms in the toxic responses will be studied using a genome wide association analysis and expression quantitative trait locus mapping is carried out to reveal phenotypic variation in the cell lines.

It is commonly considered that pathways predictive of human toxicity are restricted to closely related species (i.e. mammals). By measuring variability in gene expression among non-sentient organisms (NSOs), we will demonstrate that toxicity responses are shared in distantly related organisms. Omics experiments are planned to confirm that the genetic regions controlling variation in molecular key events observed in humans also control variation in the fruit fly. With these findings we hope to promote the use of NSOs to predict human toxicity in regulatory risk assessment.

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7.05.P-We435 From Human NAMs to Ecosystem Protection: A Key-Event–Driven Strategy for Cross-Species Toxicity Translation

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Protecting human and ecological health under a One Health paradigm requires approaches that move beyond siloed toxicological assessments. While human new approach methodologies (NAMs) generate rich mechanistic information, their relevance for ecological species remains unclear without a transparent biological bridge. Here, we present a key event (KE)–centric cross-species integration framework that operationalizes mechanistic conservation to translate human in vitro response data into ecologically meaningful evidence. Using two widely used consumer-product chemicals as case studies, we identified evolutionarily conserved KEs across endocrine signaling and cellular stress pathways. Human NAM assays were mapped to ecotoxicological endpoints based on mechanistic relevance, orthologous protein similarity, and Gene Ontology–based functional conservation. This enabled the generation of KE-aligned datasets spanning molecular, cellular, and organismal outcomes. Rather than constructing full quantitative species sensitivity distributions, we used KE-wise comparisons to evaluate how human-derived mechanistic thresholds complement or diverge from ecotoxicological sensitivity patterns. The results reveal that cross-species alignment is strongly KE-dependent rather than chemical-dependent. Nuclear receptor–mediated pathways showed consistent correspondence between human NAMs and ecological endpoints, supporting their use for filling data gaps. In contrast, stress-response KEs exhibited more divergent sensitivity patterns across taxa, highlighting the value of ecological assays in capturing species-specific vulnerabilities not reflected in human models. This KE-based strategy provides a transparent and scalable path toward mechanistic cross-species interpretation, enabling human NAMs to inform environmental protection while preserving ecological relevance. The framework demonstrates how conserved biology can anchor One Health–oriented risk assessment and illustrates the potential for mechanistic data streams to converge into integrated, interpretable evidence for chemical safety decision-making.

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7.05.P-We436 Case Study-Led Evaluation of an Integrated Human Health–Environmental Next Generation Risk Assessment Framework

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While human health and environmental risk assessment have traditionally been related but separate disciplines, there is growing recognition of the benefits of increased integration to enhance scientific robustness, efficiency,

and decision-making transparency. The evolution of chemical safety assessment is increasingly shaped by the adoption of Next Generation Risk Assessment (NGRA) principles, which prioritise non-animal New Approach Methodologies (NAMs) which are applied alongside robust and refined exposure data and models. These advances have the potential to support a more holistic approach to risk assessment.

Here, we present a novel, tiered NGRA framework designed to integrate human health and environmental safety evaluations. The framework enables cross-domain alignment during exposure and hazard characterisation, supporting unified safety decisions. Early tiers leverage in silico tools and existing data to predict properties and toxicity mechanisms relevant to both human and environmental species. Tiers 1 and 2 incorporate a battery of NAMs spanning diverse biological and taxonomic domains, generating protective points of departure (PoDs) for species of interest.

The framework's utility is demonstrated using Benzophenone-3 (BP-3), a UV filter used in personal care products. In silico predictions indicated generic toxicity mechanisms, while in vitro bioactivity data from human- and fish-derived models were assessed alongside non-vertebrate in vivo data to define safe exposure levels. Human and environmental exposures were estimated using country-specific scenarios and physiologically based kinetic (PBK) models. Molecular homology tools are employed to evaluate the evolutionary conservation of biological targets and toxicological processes to support cross-species interpretation of bioactivity data.

While the framework aims to harmonise assessment strategies, differences in exposure assessment, protection goals, and species biology necessitate domain-specific considerations. This case study-led evaluation underscores the feasibility and value of an integrated NGRA framework, supporting more comprehensive, ethical, and scientifically robust chemical safety assessments aligned with 21st-century toxicology principles.

7.05.P-We437 A new Approach Methodology Integrating Human and Ecotoxicity Data to Assess Safer Chemical Alternatives in Consumer Products

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Consumer markets are increasingly globalized, driving a rapid increase in both the quantity and diversity of chemicals used in products. As a result, human exposure to chemicals from consumer products has become widespread and is often underestimated. Moreover, inadequate post-use management and the absence of ecosystem-centric policies contribute significantly to environmental pollution; for instance, chemicals from packaging materials and plastics are now pervasive across ecosystems. Therefore, it is essential to evaluate the risks posed by marketed products using new approach methodologies (NAMs), which can integrate human toxicity and ecotoxicity data to enable the identification and recommendation of safer chemical alternatives for consumer applications. In this study, we curated the chemicals present in the consumer products marketed in Korea. We then integrated human toxicity and ecotoxicity data for these chemicals from publicly available platforms such as ToxCast, eChemPortal, EnviroTox, and other resources to understand their potential hazards to human health and the ecosystem. A cheminformatics-based approach was employed to group these chemicals according to their structural features, scaffolds, and physicochemical properties, combined with NAM based toxicity endpoints. Additionally, we employed cross species sensitivity distribution (SSD) modelling to assess associated risks across different species and species groups. To further classify products, we integrated toxicity data with the United Nation's Globally Harmonized System for classification and labeling (GHS) enabling differentiation between data-informed safer and non-safer products. Thereafter, we performed life cycle assessment (LCA) for the identified non-safer products to evaluate their impacts throughout their life cycle. Finally, we proposed safer chemical alternatives by comparing our findings with the US EPA safer chemical ingredients list and other safer chemical initiatives across the globe. In sum, our findings highlight the value of integrating human and ecotoxicity data within the NAM frameworks to identify safer chemical alternatives, contributing to global initiatives for chemical safety and sustainability.

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7.05.P-We439 Theoretical equations and statistical regressions for the median lethal and hazardous aqueous concentrations (LC50, HC50) and oral doses (LD50, HD50) as a function of octanol-water partitioning *K_{ow}*

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Setting: Over the last decades, anthropogenic pressures on the environment have dramatically intensified and diversified. Nowadays, we have to deal with 100,000+ pollutants, sites and species.

Relevance: While models are available, application to thousands of cases is severely limited by data gaps due to financial, ethical, disciplinary and other constraints. In particular, exchange of data between ecotoxicology and human toxicology is restricted to a comparison of a couple of QSARs for LC50 in fish and LD50 in rodents.

Objectives: The aim of the present study is obtain theoretical and empirical relationships for common assessment endpoints, viz. single-species median lethal (LC50) and multi-species median hazardous (HC50) aqueous concentrations for aquatic species as well as oral doses (LD50, HD50) for rodents as a function of chemical properties (esp. Kow, m) that allow for a quantitative mechanistic interpretation. more from linear to non-linear relationships, from minimum to excess toxicity, from single- to multispecies endpoints and from concentrations to doses.

Methods: As we aim to cover many substances and species, we rely on generic rather than specific properties for our theoretical analysis, in particular molecular mass and octanol-water partition ratios. To reduce variability due to different conditions we used curated toxicity data. We assigned one mode to each chemical based on the information provided by models and databases based on the following procedure. We calculated intercepts, slopes, explained variance and significance for 11 common modes of action.

Results: Empirical single-species (LC50) and multi-species (HC50) concentrations were mostly within the theoretical envelope. With some exceptions, median lethal water concentrations for single- LC50 and multiple-species HC50 decreased statistically significantly as a function of the octanol-water concentration Kow. Slopes were steep and intercepts were high for some compared to other modes of action. Lethal doses LD50 and HD50 were (near-)independent of octanol-water partition ratios Kow.

Discussion: The theoretical and statistical relationships allow comparison of data for aqueous exposure of cold-blooded aquatic species and oral administration of rodents in a consistent and mechanistic way, rather than ad hoc correlations reported in the past. These relationships provide a framework to further integrate ecotoxicology and human toxicology in an empirical and theoretical sound way.

7.05.P-We440 Next-Generation Risk Assessment Application for Potential Endocrine Disruptor Evaluation in Consumer Chemical Products

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Under the Korean Consumer Chemical Products and Biocides Safety Control Act (K-BPR), which began in 2019, the household chemical products subject to safety confirmation have been reduced from the existing 43 items to 30 items, as of December 2025. This change reflects the regulatory transition in which several categories—such as disinfectants, algacides, and repellents—will be shifted to the biocides approval regulations. Among the remaining 30 product categories, several household chemical products—such as laundry detergents and fabric softeners, cleaners, and deodorizing agents—continue to be included, and these products are used frequently in everyday life. In this study, we identified potential endocrine-disrupting chemicals (EDCs) based on household chemical products with verifiable ingredient information. We then examined the functions and roles of these substances within the products and categorized the level of concern according to their potential for exposure. The substances were initially prioritized using machine learning models designed to screen chemicals for their developmental and reproductive toxicity (DART) potential, through which candidates with possible endocrine-disrupting (EDCs) activity were identified. The results were categorized into two major groups: preservative biocidal active substances and other ingredient chemicals. For each classified substance, endocrine-disrupting potential was further evaluated using NAMs-based evidence obtained from the Tox21 database. In addition, exposure pathway information was collected and grouped according to product use patterns within the relevant regulatory context, enabling a systematic classification of substances that require further assessment. This study not only provides foundational information for identifying substances with potential endocrine-disrupting (ED) effects in household chemical products regulated as safety-confirmation items under the Korean biocides framework, but also demonstrates the applicability of next-generation risk assessment (NGRA). By presenting a case study that applies NAMs-based screening approaches in situations where available ED-related information is limited, the findings suggest the potential regulatory utility of NGRA methodologies.

7.05.P-We441 Estimating Combined Risk of Tetramethylammonium hydroxide (TMAH) and Co-Solutes in River Water

India is rapidly expanding in semiconductor chip manufacturing to push for the country's robust and sustainable technological infrastructure. However, this expansion also comes with environmental issues, which need proper mitigation and management to ensure long and sustained growth. This study focuses on the major pollutants of this industry sector, investigating the human risk assessment of these pollutants, interaction between them, and treatment strategies.

Semiconductor manufacturing releases toxic tetramethylammonium hydroxide (TMAH) and SiO₂ nanoparticles along with other heavy metals used for doping and other specific properties and PFAS coming out as byproducts. CMP processes generate high-stability nano-colloids. Environmental and human health risks remain insufficiently characterized. TMAH is a major pollutant; being a quaternary ammonium compound, its TMA⁺ cation is responsible for neurotoxicity, has a low LD50, and causes acute neurotoxicity. SiO₂-NPs exhibit contradictory toxicity data and have high environmental persistence. Combined presence of these pollutants in wastewater complicates treatment, raising the need for toxicology-based treatment optimization. Treated water from the treatment plants of these industries is being released into nearby water bodies, and the residual concentration of these pollutants should be monitored.

The plan is to first quantify acute human toxicity for TMAH using interspecies scaling and effluent concentrations. Multiple exposure pathways are to be considered for the interaction of humans with the pollutants in the water bodies. Risk estimation for TMAH and other co-solutes will be calculated for all the different exposure pathways to achieve a holistic approach to monitoring the risk for these pollutants. TMAH and its interaction with the ecological conditions of the region are to be considered to create ecologically sensitive zones. Then the toxicity indices of SiO₂-NP and major heavy metals coexisting with TMAH are to be compiled and analyzed. Data models for both acute and chronic toxicity studies will be prepared. Based on these combined risk assessment studies, a risk-treatment selection model is to be developed.

These model studies will help us make better assessments of environmental and ecotoxicological implications and identify regulatory inconsistencies. Based on the studies, if the risk is considerably higher, then specific targeted treatment methods will be developed and explored.

7.06.P Exploring Planetary Boundaries and Transformation Pathways for Novel Entities

7.06.P-Th300 Environmentally Safe and Just Pharmacy: A Framework and Action Plan for Operating Within the Earth System Boundary for Novel Entities

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Pharmaceuticals are vital for human and animal health, yet their widespread release into the environment contributes to the transgression of the "Novel Entities Planetary Boundary" and poses a significant threat to global ecosystems. This study introduces the concept of an "Environmentally Safe and Just Pharmacy," which integrates environmental protection and social equity, aligning with the "Safe and Just Earth System Boundaries" framework. We propose six overarching criteria and fifteen subcriteria that, if met, would ensure that pharmaceutical design, production and use do not contribute to exceeding the Novel Entities boundary. Based on a comprehensive review of existing data and knowledge, we conclude that current global practices for pharmaceuticals fail to meet many of our proposed criteria. The key findings are that environmental considerations are not systematically integrated into pharmaceutical design, manufacturing practices cause disproportionate pollution in vulnerable regions, and the continuous input of pharmaceuticals leads to widespread and persistent environmental contamination. We also confirm the significant contribution of pharmaceutical pollution to the global antimicrobial resistance crisis. To address this critical issue, we present a ten-point roadmap detailing necessary actions for a just transition to a sustainable future. The roadmap calls for global cooperation and includes strategic investments in sustainable molecular design, strengthening environmental risk assessment, improving wastewater treatment infrastructure, promoting responsible disposal and circular economy principles, and ensuring equitable access to pharmaceuticals for all societies. This

framework provides a qualitative foundation for future efforts to bring pharmaceuticals into an Environmentally Safe and Just operating space.

7.06.P-Th301 Methods to Assess Planetary Boundaries for Chemical Pollution - Interim and Future Approaches

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The scientific literature indicates that chemical pollution may be threatening the global environmental conditions that have supported life throughout the Holocene. This threat could push Earth out its safe operating space, thereby breaching planetary boundaries. At the same time, there are large data and methodological gaps that inhibit our understanding of the full scale and nature of the environmental risks and impacts from chemicals and chemical pollution. The hazards and exposure for the 350,000+ chemicals used globally have only been assessed for a small fraction. The toxic effects of many chemicals on plants, animals, and humans are unknown.

Previous assessments of planetary boundaries for chemical pollution (e.g. Persson et al., 2022) conclude that planetary boundaries are exceeded because the rate of chemical safety assessment and monitoring cannot keep up with the rate of production and environmental releases. To date, no fully quantitative methods to assess planetary boundaries for chemical pollution have been applied at a global scale. Moreover, previous work generally looks at the uncertainties surrounding chemical risks and impacts, rather than evidence of disruption to Earth system functioning.

WSP is currently leading a study for the European Commission with three key components:

1. Reviewing existing methods for assessing planetary boundaries for chemical pollution.
2. Developing options to quantitatively define and assess planetary boundaries for chemicals, including methods which are not currently feasible based on available data and maturity of methods, but could be feasible in the future.
3. Developing and applying interim methods for an assessment of planetary boundaries for chemicals. These methods are based on existing data, without the need for new complex models.

The review of existing methods has been completed and draft options for methods have been developed. These options will be further refined through collaboration across the project team and with a scientific roundtable of experts. The study is due to complete in May 2026, and therefore the results are expected to be available to present at the SETAC meeting.

The output of the study is intended to further the concept of planetary boundaries for chemical pollution and influence future research and policy communication.

Disclaimer/Disclosure:

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7.06.P-Th302 Equity and Sustainability: Broader Implications of Downscaling Planetary Boundaries

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As planetary boundaries (PB) gain traction as a framework for guiding global absolute sustainability, the challenge of translating these boundaries to actionable targets at national, sectoral, or company levels, known as downscaling, has become increasingly urgent. Therefore, this research aims to examine broader sustainability implications of applying different downscaling overarching principles, including equal per capita, sovereignty-based, capability-based, needs-based, historical responsibility, and ecological capacity-based approaches. Through synthesis and analysis of these overarching principles, we identify the broader sustainability implications of using these principles for downscaling PBs. While each overarching principle offers distinct ethical and operational advantages, their application raises complex trade-offs related to equity, governance, and systemic coherence. They may reinforce or undermine broader sustainability goals, such as intergenerational justice, global cooperation, and resilience across socio-ecological systems. We argue that uncritical application

of any single overarching principle risks fragmenting responsibility, exacerbating inequalities, or overlooking local contexts and systemic interdependencies. Our findings highlight the value of considering hybrid, context-sensitive approaches that balance fairness with feasibility, and global ambition with local relevance. Our research also draws on aspects that are not considered by these principles when downscaling PBs, including resource exchange (e.g., flows of physical resources and money), global flows, and cross-boundary effects, different local and regional biophysical limits, and intergenerational equity. This contribution aims to inform the development of integrated sustainability strategies that are both scientifically grounded and socially legitimate, supporting the pursuit of sustainable product and system innovation within planetary limits.

7.06.P-Th304 Getting to the Boundary: Collecting and Mapping Chemical Concentrations Globally

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Novel entities are increasingly recognized as significant drivers of the triple planetary crisis of climate change, biodiversity loss, and chemical pollution. One way to address this has been through the planetary boundaries framework, first introduced by Rockström and colleagues in 2009, which has been an effective means of communicating the urgent need to maintain the biophysical stability and resilience of the Earth system on which society depends. Among these boundaries, chemical pollution, renamed “novel entities” by Steffen et al. (2015), remains a boundary that has yet to be quantitatively defined.

Building on Rockström et al.’s (2023) refined framework of “safe” and “just” boundaries, this poster presents recent efforts towards defining the “safe” (biophysical) boundary for novel entities, focusing on chemical pollutants.

To capture the ecotoxicological impact of chemicals at a global scale, as a proxy for Earth system disruption, we compiled a harmonized, global database of measured chemical concentrations across multiple environmental media (including air, surface water, groundwater, soil, biota, and sediment) from public databases and scientific literature, building off Isaacs et al.’s (2022) multimedia monitoring database. To support long-term, large-scale analyses, our open-access database was built in PostgreSQL, which enables efficient management of relational data, easy integration of new data, as well as extraction of chemical identities, environmental media, and geospatial attributes across millions of records.

We compared these concentrations against vetted ecotoxicological benchmarks collected from 37 countries to calculate unsafe exceedances expressed as risk quotients. Aggregating these data from regional to global scales enables identification of priority pollutants and geographic hotspots, which we visualize through spatial maps. This assessment provides the foundation for a meta-analysis aimed at informing the status of chemical pollution relative to a “safe” planetary boundary.

Disclaimer/Disclosure:

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7.06.P-Th305 Mapping Global Burdens of Novel Entities in People

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Chemical pollution has been recognized as one of the major drivers of the triple planetary crisis of climate change, biodiversity loss, and pollution. The planetary boundaries framework introduced by Rockström et al. (2009) identified chemical pollution as one of nine boundaries that determine Earth’s stability and resilience, delineating a safe operating space for humanity. In 2015, Steffen et al. renamed and expanded the boundary of “chemical pollution” to encompass “novel entities.” While a qualitative argument made by Persson et al. (2022) suggests that global society is exceeding the boundary for novel entities, the boundary has not yet been formally quantified.

In 2022, the planetary boundaries framework was further refined by the introduction of “safe” and “just” boundaries. Safe boundaries describe the biophysical resilience and stability of the Earth system, while just boundaries refer to the maximum pressure that can be exerted on a system to minimize significant harm to humans. This poster presents a collation and analysis of human biomonitoring data aimed at advancing the development of a just boundary for novel entities.

We assembled a database of chemical biomarkers measured in human populations across different matrices (e.g., blood, human milk) from national and international human biomonitoring initiatives such as US NHANES and HBM4EU, complemented by additional data sourced from the scientific literature with a specific focus on low-income countries and vulnerable populations. All data are maintained in a PostgreSQL database, allowing integration of new data over time.

We identified unjust exceedances by calculating risk quotients from measured concentrations in our database and vetted health-based guidance values. Aggregating these data from regional to global scales allowed us to identify priority pollutants and geographic hotspots, which we visualized through spatial mapping. The findings further highlight significant data gaps. Our assessment establishes the basis for a meta-analysis toward defining a just boundary for novel entities.

Disclaimer/Disclosure:

This research is part of the Earth Commission’s effort to define safe and just boundaries for the Earth system. The work of the Earth Commission, a program of Future Earth, is made possible through the support of Oak Foundation and the Global Commons Alliance, a sponsored project of Rockefeller Philanthropy Advisors.

7.06.P-Th306 Global Emissions of Selected Engineered Nanomaterials: Towards Defining Planetary Boundaries for Novel Entities

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Novel entities—including engineered nanomaterials (ENMs)—pose emerging challenges to Earth system resilience due to their potential persistence, toxicity, and global dispersion. While the planetary boundary framework has been proposed to define safe operating spaces for such substances, quantifying the boundary for ENMs remains elusive due to data gaps and methodological limitations. This study advances the absolute environmental sustainability assessment of ENMs by integrating global emission estimates with a removal rate model for nanomaterials in wastewater treatment systems.

Building on AquaQuant, a globally differentiated wastewater emission model developed in previous work, we estimate emissions for nano-silver, nano-titanium dioxide, and carbon nanotubes based on production volumes, uses, and regional wastewater infrastructure characteristics. Removal efficiencies are derived from literature, coupled with treatment technology distributions, and finally applied at a global scale, enabling an approximation of ENM discharge from wastewater to aquatic and soil environments.

The results are contextualised within the planetary boundary framework for novel entities and will aim to provide preliminary insights into whether current ENM emissions may exceed estimated safe thresholds. We discuss the implications for regulatory science, the need for harmonised monitoring approaches, and the importance of integrating diverse knowledge systems to advance boundary-setting for novel entities.

This work supports the development of an emission inventory by providing a scalable modelling approach that can link global emissions to human and ecosystem impact. It also highlights the importance of including vulnerable regions in global assessments, where data gaps may mask disproportionate exposure and risk.

Disclaimer/Disclosure:

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7.06.P-Th307 Effect of anthropogenic nanoparticles on the fate of natural suspended particulate matter

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Anthropogenic nanoparticles (ANPs), whether engineered or incidentally produced, are increasingly released into marine systems, yet their influence on Earth system processes remains poorly understood. Because suspended particulate matter (SPM) plays a central role in nutrient and carbon cycling, it is essential to know whether ANPs can modify the settling rate of SPM. This knowledge is key to defining a safe planetary boundary for ANPs.

This study examines whether, and how, ANPs can alter the settling rate of natural marine SPM. To do so, three model ANPs were selected for their abundance in the natural environment : nanoplastics, nano-black carbon, and titanium dioxide nanoparticles. First, their impact on SPM settling rate was measured using freshly collected SPM from Arctic waters, during shipboard experiments. Second, complementary laboratory studies assessed interactions between ANPs and components of SPM (polar microalgae and resuspended sediments) through measurements of adhesion forces, aggregation behavior, and changes in particle size.

Results show that nanoplastics accelerated settling of SPM by nearly 30 percent, while titanium dioxide nanoparticles produced a more moderate impact. However, nano-black carbon had minimal effect. Early mechanistic results suggest that enhanced settling is not primarily driven by the bridging of particulate matter by nanoparticles but may instead relate to the formation of large nanoparticle aggregates or the presence of residual micrometric particles in the ANP suspensions.

These findings indicate that certain novel entities can modify physical processes that regulate carbon and nutrient transport, highlighting a pathway of impact that extends beyond toxicity. Understanding such mechanisms is essential for assessing global-scale risks, informing the development of planetary boundaries for novel entities.

7.07.P Health and Well-Being Effects of Blue Spaces: The Ocean-Human Health Nexus in an Ocean Under Stress

7.07.P-Th308 The Nasal Microbiome as Exposome Biomarker in Belgian Coastal and Inland Inhabitants

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Sea spray aerosols (SSAs), generated by bursting bubbles from breaking waves, contain a complex mixture of inorganic salts, marine microbiota and bioactive molecules. According to the “old friends” hypothesis, exposure to diverse microbiota at low concentrations may support immune system regulation rather than cause disease. At the same time, during Harmful Algal Blooms, SSAs have been shown to contain high concentrations of marine toxins leading to harmful respiratory health effects. Therefore, this study aims to identify which marine microbiota are present in inhaled SSAs, and to determine how SSA exposure shapes the human nasal microbiome. We seek to evaluate their potential as exposome biomarkers of coastal environmental exposure within a One Health framework.

We recruited 62 healthy adults (18–50 years) in Belgium divided into two groups: individuals living at the coast (>1 year) and inland (>1 year). Nasal swabs of the participants were collected at four time points over eight weeks, alongside weekly sea air samples. DNA was extracted from the nasal swabs as well as the sea air samples and Illumina sequencing was performed. Climate and GPS data were integrated to explore associations with nasal microbiome composition.

Results reveal distinct 16S nasal microbiome profiles between coastal and inland groups, with shared genera typical of human nasal microbiota (24%) and unique genera specific to each group (17% for the coastal and 11% for the inland group). Importantly, genera overlapping between sea air samples and coastal nasal swabs (13%) suggest that marine microbiota are present in the nasal cavity of coastal residents.

These findings suggest that living near the coast may lead to inhalation of marine microbiota, potentially influencing human health. Functional analysis of these genera is ongoing to assess their potential impact on human health. The temporal design allows investigation of microbiome stability, weather-driven fluctuations, and the role of individual behavior (e.g., time spent outdoors, physical activity) in shaping the nasal exposome.

This study supports the nasal microbiome as a non-invasive exposome biomarker to assess environmental exposure to natural ecosystems. In line with One Health, this work highlights how environmental microbial dynamics at the ocean-atmosphere interface can shape human health outcomes—underscoring the need to protect both ecosystem and respiratory health in an era of rapid environmental change.

7.07.P-Th309 Immunological responses to marine aerosols: the influence of bacterial abundance and endotoxin levels

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It is increasingly recognised that “blue spaces” promote human health and well-being through psychological, physiological, and social benefits. Beyond these effects, sea spray aerosol (SSA) exposure may provide an additional pathway linking the ocean to human health. These aerosols contain diverse microorganisms and biogenic molecules that are presumed to interact with the human immune system, yet direct evidence of such interactions is lacking.

This study investigates whether human immune cells recognise and respond to marine bacteria present in SSA and whether that exposure may confer regulatory effects. Natural SSA were collected along the Belgian coast and complemented by laboratory-generated SSA. Bacterial and endotoxin concentrations were quantified, and human reporter cell lines were used to assess the activation of key immune receptors and transcription factors, including toll-like receptors (TLR4, TLR2/6) and the transcription factors NF- κ B and IRF. Concentration–response relationships were established, and assays combining a challenge test with SSA pre-exposure were used to examine potential immune priming effects.

SSA exposure induced dose-dependent activation of all immune pathways, with receptor activity correlating with bacterial and endotoxin levels. Pre-exposure experiments revealed non-linear effects: low-level SSA exposure tended to dampen responses to subsequent pathogen challenge, while higher exposures enhanced activation. This pattern suggests a complex balance between immune regulation and potential pro-inflammatory stimulation.

Our findings provide the first evidence that human innate immune cells detect and respond to marine aerosol microorganisms. These results suggest that low-level exposure to SSA may contribute to the health benefits associated with coastal environments, while high exposure levels could carry inflammatory risks. This work advances understanding of the biological dimension of ocean–human interactions and highlights the need to consider marine microbial aerosols in assessing coastal health effects.

7.07.P-Th310 Emotional Responses to Coastal and Urban Environments: an Immersive Video Experiment

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Although evidence for the health and well-being benefits of coastal environments is growing, the impact of these settings on affect and emotions remains unclear. Moreover, coastal areas are highly diverse and include distinct sub-environments that may evoke different emotional experiences. Understanding how the coast and its various sub-environments shape emotional responses is crucial for uncovering their therapeutic potential and informing evidence-based coastal planning. In this study, we conducted a randomized crossover experiment in which 60 participants watched eight sub-environmental videos within an immersive setting under two conditions: four coastal videos and, as a control condition, four urban videos. The coastal videos depicted a breakwater, the beach, the dunes, and the dike, while the urban videos consisted of two church scenes and two videos of aesthetically pleasing façades. For each video, participants provided four emotional state reports—two during the video and two post-exposure. During viewing, participants continuously reported their real-time valence and arousal level using a joystick, reflecting two core dimensions of affect. Following each video, they rated the degree to which they experienced two complex emotions: awe and connectedness to the environment. The experiments have been completed, and all data have been collected. In line with our expectations, preliminary results indicate that videos in the coastal condition yielded higher scores for valence, awe and connectedness to the environment, but also for arousal, where we initially expected lower scores compared to videos in the urban condition. The combination of more positive valence and higher arousal when watching the coastal videos suggests that participants felt more elated and happy compared to the urban videos. When

examining specific sub-environments, the dike video consistently produced lower scores across all outcome measures relative to some or all other coastal videos. A possible explanation for this is that the dike video was perceived as a less natural environment compared to the others, as it was the only coastal video that included built elements and the only one in which people were clearly passing by. These findings highlight the importance of distinguishing between coastal sub-environments when assessing their emotional impact, and they provide insights that may guide policy makers in the design of coastal spaces.

7.07.P-Th311 Marine Mammals Stranded In The Basque Coast Under The One Health Approach:

Anisakis Sp. And Avian Influenza

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When discussing the importance of marine mammals within the One Health perspective, among the main factors of interest are the zoonotic diseases and the presence of parasitic agents that could impact human activities and/or health. In the case of the zoonotic diseases the infection by influenza A virus has been frequently detected in marine mammals causing different grade mortality events. In the last years, the highly pathogenic avian influenza H5N1 virus has been rapidly expanding and infecting a new variety of avian and mammal species, including marine mammals, resulting in high mortality rates among pinnipeds and cetaceans. Among the most studied parasitic agents affecting marine mammals are the worms belonging to the *Anisakis* genus, as they infect intermediate hosts of commercial and gastronomic interest. These ubiquitous nematodes, well known gastrointestinal parasites, which use marine mammals as final hosts in their life-cycle, infest these when fish and cephalopods with their larvae are consumed. The aim of this work was to investigate the presence and impact of the mentioned parasites and virus in marine mammals stranded in the Basque Coast. For that purpose the stomach content of 21 specimen of 3 species of odontocetes (*D. delphis*, *S. coeruleoalba*, *P. phocoena*) stranded in the Basque Coast (2023-2025) were analysed to determine the prevalence and mean abundance of *Anisakis* sp. Other samples including hyssop collection (oral cavity, blowhole, anus) and frozen tissues (brain, lung, muscle, intestine) from 39 marine mammals were studied to evaluate the influenza A virus infection in the Basque coast from 2012-2024. Samples were collected by the Stranded Cetacean Network of the Basque Country (SAREUS) and were archived in the Biscay Bay Environmental Biospecimen Bank of the Plentzia Marine Station. Present results indicate that all species were infested with *Anisakis* sp. (prevalence ranging from 50-100%), massive loads being recorded mainly in *S. coeruleoalba*. In conclusion, these mammals can be considered as indicators of *anisakis* loads affecting fisheries and hence, human health. On the contrary, all samples were negative for influenza. Despite the negative results obtained, the continuation of the monitoring of the presence of *Anisakis* sp. and influenza virus in marine mammals, together with possible health affection due to the presence of environmental stressors (i.e. pollutants, global warming, interaction with human activities).

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7.07.P-Th312 Strategies for Healthy Ageing: Physiological, Psychological and Cognitive Responses to Coastal vs. Urban Exposure in Older Adults

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With population ageing accelerating worldwide, maintaining functional ability through preventive health strategies is becoming increasingly important. In this context, exposure to natural environments has emerged as a promising contributor to physical and mental well-being. Although observational studies suggest multiple health benefits of coastal environments, experimental evidence remains limited. Research is particularly scarce on physiological and cognitive effects in older adults, as well as on distinctions between passive and active

exposure. To address these gaps, this field study examined how coastal versus urban exposure affects physiological, psychological and cognitive outcomes in older adults, comparing seated and walking conditions. In a within-subject cross-over design, 48 older adults (60–86 years, 54% male) completed both a coastal and urban exposure session, each consisting of a 15-min seated phase, followed by a 30-min walk. Physiological measures (salivary cortisol, heart rate and heart rate variability) were collected before, during, and after exposure. Self-reported stress and mood, as well as cognitive performance (visual attention and processing speed) were assessed before and after exposure. During recovery (20 min after the walk), sustained reductions in cortisol were observed only after coastal walking (–15%, $p = .008$), and parasympathetic activity remained elevated after coastal walking but decreased after urban walking (–13%, $p = .043$). Coastal walking was also associated with an overall reduction in perceived stress (post-pre contrast: –6.66, $p = .039$), improved visual attention (+38.91 test score points, $p < .001$) and larger gains in processing speed (+7.48 test score points, $p < .001$). Mood did not change significantly (all $p > .05$), as baseline positive mood was already high and negative mood was low, limiting the potential for further change. These findings indicate that coastal environments may provide added restorative value beyond physical activity alone, since coastal walking was associated with stronger physiological, psychological, and cognitive benefits than urban walking in older adults. Coastal walking may therefore represent an accessible, low-cost, and scalable strategy to support healthy ageing. Future research should clarify dose-response relationships, including the optimal duration and frequency of coastal exposure needed to achieve these health benefits.

7.07.P-Th313 A transdisciplinary framework to accelerate research on ocean-human health connections

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The ocean has long been associated with human health benefits and a general sense of wellbeing. Despite multiple historical and cultural remnants, such as sea sanatoria and thalassotherapeutic centra that proclaim an intrinsic positive connection between the ocean and human health and the ocean, we still lack comprehensive, evidence-based mechanistic and epidemiological data for these health effects.

The Ostend Working Group on Ocean-Human Health (OHH) presents an OHH framework to assess how ocean health relates to human health. The OHH framework is built around four key components: (a) ocean stimuli; (b) interaction with ocean stimuli; (c) individual sensing and processing; and (d) individual and collective health outcomes.

The OHH framework allows a holistic view on OHH research and integrates findings and perspectives from biological sciences, psychological sciences and social sciences into a transdisciplinary approach. Moreover, the OHH framework offers researchers a global OHH context and a systematic research approach that facilitates the harmonization of ad hoc research questions and associated hypotheses towards the shared objective of understanding the pivotal role of the ocean on human health.

The OHH framework is designed to support scientists, policymakers, and the public in investigating the intrinsic connection between ocean and human health. By promoting a holistic approach that prioritizes respect and care for the oceanic spheres, such as through integrated policies, it ultimately contributes to healthier ecosystems and communities.

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<https://www.vliz.be/en/sea-good-cause>.

7.07.P-Th314 One Health case study at the bay of Plentzia (Basque Country)

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This study explores the application of the One Health paradigm in the Plentzia estuary, aiming to integrate environmental, human, and ecosystem health through a Living Lab approach. The project was co-designed by researchers from PiE-UPV/EHU and BC3 to address complex socio-ecological challenges in coastal systems. Its main objectives were: (i) to investigate the dynamics of environmental pathogens and their relationship with climate change drivers and pollutants; (ii) to develop an extended One Health conceptual framework that captures interconnections between coastal pathogen changes and human, animal, and plant health; and (iii) to establish a One Health Living Lab (OHLL) as an open, participatory space for innovation and governance.

A comprehensive monitoring program was implemented in 2023–2024, including monthly and seasonal sampling in the estuary and bay. Water column profiles, chemical analyses, eDNA sequencing, and biological sampling (zooplankton, phytoplankton, phytobenthos, and sentinel species) were conducted alongside socio-economic assessments and wastewater effluent monitoring. Results revealed significant seasonal patterns: zooplankton abundance peaked in spring and summer, while phytoplankton composition varied more between bay and estuary than by depth. Notably, blooms of *Ostreopsis cf. siamensis* exceeded alert thresholds for the first time, coinciding with water temperatures above 20.5°C. *Vibrio* diversity and pathogenicity were highest in summer. Antibiotic resistance was prevalent below the halocline, with 52% of isolates being native marine bacteria, 24% potentially pathogenic. Biomarker analysis in oysters established reference levels for stress indicators, showing seasonal variability linked to salinity, temperature and contamination. Chemical monitoring revealed pharmacological compounds and pesticides in WWTP effluents, with seasonal trends aligned with rainfall. Socio-economic studies highlighted demographic patterns and beach usage peaks during summer.

The extended One Health framework integrated the DPSEEA model with participatory cognitive mapping, enabling visualization of interdependencies among environmental, biological, and social health determinants. Monitoring results fostered engagement with hospitals, public health agencies, water authorities, NGOs, and citizens, strengthening governance and knowledge transfer, and positioning the OHLL as a collaborative platform for sustainable innovation.

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7.07.P-Th315 Microbial Hydrocarbon Degradation Potential of the Baltic Sea Ecosystem

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The Baltic Sea receives petroleum hydrocarbons from various point sources. The degradation of these contaminants in the environment is typically facilitated by a variety of microorganisms that possess a range of genes and metabolic functions related to the degradation of various hydrocarbon substrates. However, our understanding of natural attenuation and the microbial capacity to degrade these contaminants within the Baltic Sea ecosystem remains limited. In this study, we compiled metagenomes from the benthic and pelagic ecosystems across the Baltic Sea to identify microorganisms and characterize their genes and metabolic functions involved in the degradation of hydrocarbon compounds. Known hydrocarbon-degrading phyla, i.e., Pseudomonadota, Myxococcota A, Actinomycetota, and Desulfobacterota, were identified within the Baltic Sea metagenome-assembled genomes (MAGs). Notably, 80% of the MAGs exhibited multiple hydrocarbon degradation gene annotations (>10 reads per kilobase million). Aerobic degradation was the predominant pathway for hydrocarbon degradation across environmental samples. Hydrocarbon degradation gene abundances varied among samples and Baltic Sea subbasins, with long-chain alkanes and dibenzothiophene compounds being the preferred substrates. Species richness and diversity of both benthic and pelagic microorganisms positively correlated with hydrocarbon degradation gene diversity, with the pelagic ecosystem exhibiting significantly higher richness and diversity compared to the benthic ecosystem. Additionally, the composition of

the hydrocarbon degradation genes across the Baltic Sea subbasins was influenced by oil spill history, with areas that experienced higher spill volumes showing lower microbial diversity, suggesting potential enrichment of specific hydrocarbon degraders. Among the environmental factors assessed, depth played a significant role in shaping the composition of genes involved in hydrocarbon degradation within the Baltic Sea. This knowledge will aid in understanding the natural capacities of microbial communities, potentially linked to the natural attenuation of hydrocarbon pollutants in the area. Insights into microbial degradation potential can enhance predictions of petroleum pollutant persistence and accumulation, support mitigation strategies for marine pollution, and reveal the ecological resilience of native microbial communities in marine ecosystems.

7.08.P Holistic Sustainability Assessment: Innovative Approaches for Integrating Environmental, Societal, Health, and Economic Analysis for Decision-Making

7.08.P-Th316 A First-of-Its-Kind Ontology Supporting Safe and Sustainable by Design Decision-Making in the Plastic Value Chain

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1. Background

Implementing Safe and Sustainable by Design (SSbD) principles in the plastic value chain demands coherent integration of heterogeneous information covering safety, environmental, economic, and social dimensions. However, the data underpinning these evaluations remain fragmented across databases, processes, stakeholders, and life cycle stages.

2. Objective

This work presents one of the first ontology-based frameworks specifically engineered to formalise the complex life cycle and assessment knowledge required to operationalise SSbD principles in the plastic value chain. The ontology provides a semantic architecture that structures domain information, integrates diverse type of data, and enables coherent evaluation across the four dimensions.

3. Methods

The ontology was developed using a modular approach aligned with the European SSbD Framework, combining a top-down translation of SSbD principles with bottom-up input from sustainability experts. It structures the plastic value chain into core domains: Component System, Life Cycle Phases, Processes, Subsystems, and an SSbD Assessment Framework, each formalising entities and relationships to represent material flows, process logic, and evaluation needs. Implemented using the Web Ontology Language (OWL), the model defines relevant classes, properties, and constraints, while a dedicated assessment module encodes indicators, system boundaries, and Technology Readiness Level (TRL) rules to adjust evaluation scope based on technological maturity.

4. Results

This ontology provides a harmonised representation of the plastic value chain, linking material flows, processes, and SSbD indicators in a coherent structure. Its TRL-based assessment logic enables evaluation depth to adapt to technology maturity, supporting both early-stage screening and comprehensive analysis. This structure improves data interoperability and supports integration into decision-support tools for SSbD implementation.

5. Conclusions

This first-of-its-kind ontology addresses the fragmentation of SSbD-relevant data in the plastic value chain by unifying life cycle and assessment information into a coherent semantic framework. By providing a structured backbone for data interoperability, the ontology supports the development of decision-support systems that can automate SSbD evaluations, guide material and process selection, and improve transparency in sustainability-driven design.

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7.08.P-Th317 Material Bottlenecks in the Green Transition: The Case of Resource Criticality and Circular Design in PEM Electrolyzers

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Green hydrogen will play a key role in the energy transition. In this regard, Proton Exchange Membrane Electrolyzers (PEMEL) are an important solution for its production, as they can track intermittent load profiles from renewable energy sources such as wind and solar. Despite their advantages, they depend on critical and strategic raw materials with a highly concentrated supply. The platinum group metals used as catalysts have the potential to act as a bottleneck during market scale-up. Alongside the imperative of sustainability, it is therefore essential for the assurance of a secure supply chain to implement R-strategies throughout the entire life cycle of PEMEL. The early market stage of PEMEL results in substantial variations in the composition of raw materials utilized, the design, and the implementation of condition monitoring of current systems on the market, which hinders the standardized implementation of R-strategies.

This work presents a comprehensive multi-criteria decision-analysis approach for identifying optimal circular strategies for PEMEL systems at various life cycle stages. Building upon the Resource States Framework, we systematically decompose PEMELs into product, part, and particle levels to map relevant R-strategies across each stage of use and end-of-life management. We propose a methodological approach combining life cycle sustainability assessment (LCSA), technological feasibility and criticality aspects, with dedicated supply chain analyses to capture the named parameters overlooked in conventional sustainability assessments.

The proposed holistic framework enables early identification of potential trade-offs among environmental integrity, economic and technological feasibility, and resource security and scarcity, thus informing policy development and industrial practice toward resilient PEMEL value chains. This facilitates the implementation of identified requirements for future economic systems, such as the implementation of a digital product passport or the targeted implementation of circular design.

7.08.P-Th318 Integration of Risk Assessment Results into a Holistic Evaluation of Remediation Solutions for Contaminated Sites – a Case Illustration at Tier 1 and Tier 2 Levels of Risk Assessment

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Europe faces many contaminated sites, with an estimated 60–70% of soils considered unhealthy. These sites often require remediation to reduce risks to human health and the environment. Yet actions targeting one contamination problem may have variable efficiencies and unintended consequences on other contaminants, soil health, ecosystem services, land use, or the local economy. Tools are needed to help decision-makers distinguish between restorative and regrettable remediation. Existing frameworks assess risks to health and environment through tiered, stepwise approaches, progressively adding detail as contamination is characterized. A key challenge is integrating risk assessment results into transdisciplinary discussions with stakeholders at all stages, including early phases when scenarios are not yet chosen and contamination is poorly known. Flexibility of the framework is essential to incorporate risk aspects from the start, update information as it is collected, and adapt to diverse national regulations across Europe. In ARAGORN, risk assessment tools are developed to provide early risk metrics that can be integrated into holistic sustainability assessments. They enable Tier 1 evaluations based on soil concentrations, with straightforward upgrading to Tier 2 using site-specific data such as soil properties or concentrations in porewater, groundwater, or plants. Tier 1 results can support stakeholder discussions to identify and prioritize parameters requiring further investigation. A remediation add-on allows parametrizing theoretical effects of remediation scenarios, enabling early screening of options and prioritization of resources for site-specific tests. Results from these tests can then refine evaluation of remediation impacts on risk indicators. The presentation will describe the methodological approach for risk assessment and indicate how outcomes are integrated into a socio-economic decision-making framework. A case study will illustrate the methodology, conditions of applicability, and limitations. These aspects will be discussed in relation to other existing tools. Finally, the potential for ARAGORN tools to be further developed and applied will be outlined, along with future work to integrate them into co-creation and transdisciplinary efforts in soil remediation,

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7.08.P-Th319 Economic Sustainability Assessment of Sea Bass Feed with Black Soldier Fly Meal: Integrating ESM, TEA, and I-O Models”

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Recent environmental policies have placed greater emphasis on the use of more sustainable products and technologies, thereby increasing the need for integrated assessments capable of guiding these decisions. In this context, insect-derived products are a sustainable alternative, but require analysis of economic, social, and environmental impacts throughout their life cycle. This study assesses the economic sustainability of sea bass feed produced with Black Soldier Fly (BSF) meal, integrating methods that complement Life Cycle Assessment to support more robust decisions.

The challenge is to understand and to analyse how an innovative product compares with a conventional alternative in terms of direct, indirect, and economic welfare effects. The work combines three approaches: the Economic Surplus Model (ESM), which estimates gains for producers and consumers; Technical-Economic Analysis (TEA), to assess financial viability; and the Input–Output (I-O) model, which calculates impacts on production, wages, and imports in the Portuguese economy. The data refer to 2025 and have been normalized to 1 ton of product.

The results show that conventional aquafeed has an economic impact of €1.73 per €1 invested, whereas BSF aquafeed has an impact of €1.58, reflecting a lower impact on the Portuguese economy in this food chain. Despite this, BSF aquafeed has a lower cost of approximately 8.85% per ton (€882.18) compared to conventional aquafeed (€967.86). Import dependency is similar: 30.16% for conventional aquafeed and 32.85% for BSF aquafeed. The impact on remuneration is also very similar, with values of around 33.64% (BSF) and 33.38% (conventional). In the ESM, aquafeed (BSF) shows greater welfare gains per ton (

7.08.P-Th320 Comparing and Prioritizing Measures for Remediating Polluted Soils: A Holistic and Integrated Framework for Socio-economic Analysis

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Soil contamination caused by single contaminants or mixtures affects both ecosystem organisms and humans. Negative impacts on ecosystems and human health can spread from local to regional, continental, or even global scales, and can last for decades or even centuries. Furthermore, soil contamination can cause high welfare losses to society, e.g. a decline in the healthy living environment for terrestrial and water organisms; reduced availability of healthy food and drinking water along with corresponding clean-up costs; increased treatment costs for certain diseases; and costs resulting from the loss of ecosystem services. Addressing soil contamination, therefore, is a multi-dimensional social challenge. Soil remediation denotes all management actions that aim to remove degradation (e.g., detoxify areas with contaminants or excess nutrients from soil and water) to achieve safe and stable environments. Remediation is then followed by restoration actions to suitably achieve the target 'reference model' of the site according to its desired long-term use. A number of technologies for soil remediation and ecological restoration have been developed. Decision-makers such as land-owners, local municipalities or stakeholders have to compare technically available remediation options and restoration scenarios, and identify the scenarios which are most appropriate.

This study introduces a holistic framework for socio-economic analysis (SEA) to compare and prioritize soil remediation and restoration scenarios. The framework provides guidance on how information about hazards and environmental and human health risks of soil pollution can be transformed into comprehensive outcomes such as positive and negative impacts, the change of ecosystem resilience, and corresponding social costs and benefits of remediation measures. This transformation process is facilitated by input from experts, local stakeholders and citizens, making SEA a co-creative process instead of a purely one-directional analysis. Furthermore, it is shown how positive impacts are to be balanced against negative impacts, and against the costs of soil remediation and restoration scenarios. SEA results in transparent decision rules in order to identify the remediation and restoration scenario which is socially (most) beneficial. Finally, the framework describes how the assessed positive and negative impacts, benefits and costs of soil remediation can be transformed into spatially explicit information.

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7.08.P-Th321 The ARAGORN Decision tree - guidance for remediating and restoring contaminated soils through co-creation and integrated risk, socioeconomic and resilience assessment

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Managing contaminated land is a complex challenge, as the best remediation or management approach is not always evident. A holistic evaluation must go beyond assessing current contamination risks to include the potential impacts of remediation strategies, life cycle considerations, socio-economic implications, and resilience to future changes such as climate variability or evolving land use. Addressing these dimensions is critical to avoid regrettable remediation and ensure long-term ecological and economic value. The ARAGORN project introduces the ARAGORN Decision Tree, a structured tool designed to guide landowners and stakeholders through this process. The decision tree integrates regulatory requirements, risk assessment, and sustainability principles into a practical framework for informed decision-making. It consists of three main phases:

- Phase 1: Pre-Assessment

- o Step 1a: Determine when to investigate soil contamination based on national regulations and practices.
- o Step 1b: Analyze boundary conditions, including intended land use, timeframes, economic constraints, and stakeholder interests.
- o Step 1c: Assess contamination status using standardized risk analysis guidelines

- Phase 2: Defining Needs and Goals

Evaluate the necessity for remediation and restoration by coordinating risk assessments with socio-economic and resilience analyses to identify benefits and objectives.

- Phase 3: Monitoring Progress

Track key indicators and socio-economic goals to ensure remediation and restoration actions remain on course.

Throughout all phases, the decision tree emphasizes alignment with EU and national regulatory contexts, stakeholder co-creation, and the use of dashboards for spatial planning and indicator tracking. By combining technical, environmental, and socio-economic perspectives, ARAGORN provides a robust decision-support system that bridges science, policy, and practice, contributing to sustainable land management and resilience.

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7.08.P-Th322 Towards Healthy Soils: A Decision Support Tool for Sustainable Remediation Practice

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Persistent and bioactive pollutants pose a major challenge to soil health in Europe, where an estimated 60–70% of soils are considered unhealthy. Despite ongoing EU initiatives aiming for healthy soils by 2050, remediation remains complex and interdisciplinary, involving stakeholders, problem owners, and regulators. Different technologies modify key parameters in distinct ways—for example, soil washing reduces total contaminant concentrations, sorbent amendments lower bioavailable fractions, and top cover systems decrease infiltration rates. Selecting an appropriate method is further complicated by site-specific constraints such as soil texture, contamination extent, and the diverse behaviours of persistent contaminant groups. So far, helpful decision-support systems for selecting adequate remediation options are missing.

In this study, we developed an Excel-based remediation tool that serves as a user-friendly, cross-disciplinary decision-support system. The tool quantifies changes in risk-related parameters, which can be manually integrated into existing human health and environmental risk assessment frameworks to estimate risks after remediation. Its database is built on a structured literature review and expert input from ARAGORN project partners. The tool covers eight remediation methods—excavation and containment, capping, monitored and enhanced natural attenuation, phytoremediation, emerging bioremediation using nanomaterials, biochar and Fe-

based sorbents, chemical reduction, and soil washing—and evaluates their effects on four contaminant groups: Per- and Polyfluorinated substances (PFAS), organochlorinated and brominated substances (OCBs), petroleum and coal substances (PETCO), and metals.

Based on site- and contamination-specific inputs, the tool identifies feasible remediation options and provides quantitative estimates of how each method influences parameters relevant for risk assessment. Users can compare how strategies affect bioavailability, emissions, or mobility for different contaminant groups before and after remediation.

This remediation add-on is designed to complement existing risk assessment tools and represents a step toward a holistic framework for soil restoration that integrates human and environmental perspectives. It supports evidence-based decision-making for sustainable soil management across disciplines.

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7.09.P Implications of Climate Change for Agriculture Ecosystems: What Does It Mean for Plant Protection Product Risk Assessments and Agricultural Practices?

7.09.P-Th324 Mitigating Climate Stress in Tea Cultivation: Monitoring Conventional vs. Eco-friendly Strategies for Environmental Risk Assessment and Crop Resilience

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Climate change poses significant threats to established agricultural systems, particularly for sensitive high-value crops like tea (*Camellia sinensis*) in Taiwan. Rising temperatures and increasing extreme weather events are shifting agroecological boundaries, challenging farming practices, and altering environmental risk landscapes regarding pest dynamics. To develop actionable knowledge, it is critical to quantify how agricultural practices, specifically conventional versus eco-friendly approaches, modify the crop microenvironment and influence environmental risks.

This study uses long-term in-situ eddy-covariance (EC) monitoring to investigate the impact of management strategies on the microclimate and energy fluxes of tea fields in Northern Taiwan. We compared a conventional operation field with an eco-friendly (organic-certified) field to assess impacts on "field-level" climate resilience. Observations reveal a stark contrast in energy partitioning: the eco-friendly field exhibited 10% lower sensible heat flux and 25% higher latent heat flux (evapotranspiration) than the conventional field. Crucially, the eco-friendly practice demonstrated superior buffering of environmental extremes; the diurnal range of canopy temperature in the conventional field was 1.6°C higher than in the eco-friendly field, indicating higher susceptibility to heat stress.

While the eco-friendly practice resulted in a higher daily soil water loss rate (0.97% d⁻¹ vs. 0.45% d⁻¹), it successfully reduced vapor pressure deficit (VPD) and temperature variation within the canopy. These differences are pivotal for risk assessment. The hotter, more variable microenvironment in conventional fields may exacerbate pest outbreaks and alter the degradation and environmental fate of plant protection products (PPPs), potentially increasing spatiotemporal heterogeneity and uncertainty in exposure and risk. Conversely, the cooler, more humid microclimate in eco-friendly fields suggests a stabilizing effect. These findings provide a scientific basis for farmers to adopt eco-friendly strategies that conserve the ecosystem and manipulate the microclimate to reduce vulnerability under changing climatic conditions.

Keywords: Environmental Risk Assessment, Eco-friendly Practices, Microclimate Monitoring, Climate Change Adaptation, Tea Cultivation

7.10.P Innovative and Transdisciplinary Approaches to Eliminate Hazardous Chemicals and Develop More Sustainable Chemicals and Materials

7.10.P-Th325 Towards a Safe, Sustainable and Sufficient Chemistry

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In October 2020, the European Commission published a Chemical Strategy for Sustainability (CSS) to better protect citizens and the environment while ensuring the competitiveness of the European chemical industry

where they proposed, among others, to phase-out uses of the most harmful substances, unless proven essential and to encourage the development of chemicals and materials which are safe and sustainable by-design (SSbD). Together with alternatives assessment processes, the essential-use concept and the SSbD frameworks are tools which could be applied to help limit the uses of harmful chemical substances and encourage the implementation of alternatives with limited impacts on the environment and human health. The main goal of this study is to systematically compare these concepts to clarify their respective scopes, methodologies, and decision-making functions to determine how they could complement each other to support a coherent, science-based decision-making process for reducing chemical risks and promoting sustainable chemical innovation within planetary boundaries by analyzing their respective guidance documents to identify potential similarities and differences between the frameworks.

Based on our findings, we believe that the different frameworks could be implemented sequentially to limit development and uses of harmful chemical substances. The effort for substitution could be focus only on functions which are necessary to the final products and society by applying the essential-use concept first. Such evaluation can be followed by an assessment of alternatives to determine whether potential safer and suitable alternatives delivering similar functions are already available. The SSbD framework can then be applied to design new chemicals or materials only if it is not the case.

We believe that such approach could be implemented in a regulatory context when restricting uses of a substance of concern. We also believe that this approach could be applied on a voluntary basis by companies seeking to (re)design their products and minimize impacts to the lowest level necessary to achieve the intended product function.

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7.10.P-Th326 Enhancing Hazard Assessment in Safe and Sustainable by Design: The Case of Mistra SafeChem

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Safe and Sustainable by Design (SSbD) is an emerging European approach aimed at guiding the development of chemicals and materials that are safe throughout their life cycle while supporting broader sustainability goals. A central pillar of SSbD is early-stage hazard assessment, yet this step is frequently limited by incomplete data and slow or resource-intensive testing. In silico tools therefore play a critical role in enabling proactive chemical design and informed decision-making.

Within the Swedish research programme Mistra SafeChem, we have developed a comprehensive in silico hazard prediction toolbox that provides calibrated and validated predictions for 35 human health and environmental endpoints using large, up-to-date datasets and uncertainty estimation supported by conformal prediction. The toolbox includes multiple machine-learning and deep-learning models and offers consensus predictions with quantified uncertainty through a user-friendly interface. Alongside model development, Mistra SafeChem has designed several SSbD case studies with industrial partners that apply these tools to real-world chemical and material innovation challenges, demonstrating practical integration of in silico hazard assessment into SSbD workflows.

To support broader SSbD implementation, we also apply established tools such as VEGA, the OECD QSAR Toolbox and other widely recognised predictive systems. These tools complement the Mistra SafeChem in silico Toolbox by enabling read-across, mechanistic exploration, applicability-domain analysis and integration of additional regulatory-relevant endpoints. Together, this suite of models enables more robust and transparent hazard evaluations.

Across both Mistra SafeChem and several EU SSbD-focused research projects—including PARC, TORNADO, BioSusTex and CheMatSustain—we investigate how in silico modelling can fill key data gaps, prioritise safer chemical alternatives and guide targeted experimental follow-up. Results show that consensus modelling strategies can improve predictive accuracy, while applicability-domain assessments reveal limitations: some case-study chemicals fall outside the coverage of current ecotoxicity models, highlighting the need for expanded training datasets and refined modelling approaches.

Overall, the combined use of complementary in silico tools provides an efficient and scalable foundation for SSbD hazard assessment, supporting reduced testing needs and accelerating sustainable chemical design.

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7.10.P-Th327 Predicting Hazard Statements Under the Classification, Labelling and Packaging Regulation Using Conformal Prediction and Molecular Representations

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Effective communication of chemical hazards is essential for protecting human health and the environment, yet many substances lack sufficient experimental data to support reliable hazard classification under the European Union Classification, Labelling and Packaging (CLP) regulation. This study addresses the challenge of predicting hazard statements (H-statements) for human health and environmental endpoints by developing non-animal computational models trained on regulatory-compliant data extracted from the European Chemicals Agency Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) registration dossiers. We constructed machine-learning models using Random Forest classifiers combined with Mondrian conformal prediction to provide calibrated confidence estimates for each prediction. Multiple molecular featurization strategies were investigated, including N-grams, molecular fingerprints, and extended connectivity descriptors, and were further combined using consensus modelling approaches based on class votes or median conformal prediction p-values. The models were trained and evaluated across ten hazard-statement groups comprising thousands of classified and non-classified substances with standardized SMILES structures. Results show that most featurization strategies produced valid conformal predictors, but ensemble consensus models consistently achieved the highest performance. At a typical significance level of 0.2, many models provided efficiencies near or above 80%, indicating a high proportion of informative single-label predictions. Balanced accuracy, sensitivity and specificity were robust across endpoints, and N-gram-based descriptors proved particularly advantageous for representing diverse chemical structures, including those containing metals and salts.

These findings demonstrate that combining conformal prediction with complementary molecular representations enables reliable and transparent prediction of H-statements under the CLP regulation. The resulting models support chemical hazard assessment, read-across applications and risk management, offering a practical in silico tool to reduce the need for new animal testing while improving accessibility to hazard information.

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7.10.P-Th328 Application of the Safe and Sustainable by Design Framework: A Case Study on Bisphenol A and Its Alternatives

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The European Commission's Safe and Sustainable by Design (SSbD) framework aims to integrate safety and sustainability considerations early in chemical innovation. Bisphenol A (BPA) and two alternatives, Bisphenol AP (BPAP) and Isosorbide, were selected as substances in this fictitious case study to evaluate the framework's applicability in polycarbonate and epoxy resin applications.

The challenge was to determine whether existing tools could support decision-making under conditions typical of early innovation, where data availability is limited. To address this, 22 tools included in the PARC SSbD toolbox developed in the PARC project were tested across the following parts of the assessment: hazard identification, worker and environmental risk, consumer risk, environmental sustainability, and socio-economic impact. Tools were mapped to innovation stages to identify their suitability for early versus late development phases.

Methods included predictive modeling for hazard endpoints, exposure estimation for production and use phases, life cycle assessment for environmental impacts, and social life cycle assessment and lifecycle costing for socio-economic risks, emphasizing the importance of supply-chain considerations. The approach emphasized minimal data requirements and integration of multiple tools into model pipelines.

Results show that hazard assessment tools can provide early screening with limited input, while exposure models for production and application phases vary in complexity and conservatism. Environmental sustainability assessments highlight significant differences in life-cycle impacts among substances, and socio-economic analysis reveals strong regional influence on social risk outcomes. Across the full assessment, the case study demonstrates that tool applicability and reliability depend on data availability and integration strategies.

In conclusion, the findings confirm that the EC SSbD framework can guide early-stage innovation and provide actionable insights for chemical substitution. Importantly, the results inform improvements to the PARC SSbD Toolbox, enhancing its usability and relevance for innovators and regulators seeking safer and more sustainable solutions.

7.10.P-Th329 Using Machine Learning Approaches to Understand Microbial Metabolism of Pharmaceuticals within the Environment to Facilitate Design for Degradation in Medicinal Chemistry
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Within Green Chemistry, the concept of 'benign by design' is of particular interest, with the 10th principle of Green Chemistry, 'design for degradation' suggesting that there should be effort put into designing chemicals and products to degrade at end-of-life. Pharmaceuticals in the environment (PiE) are considered a global threat, both to ecological and human health, and therefore require an environmental risk assessment (ERA) at the point of EU marketing approval. However, the degradability of pharmaceuticals in the environment is not currently considered in R&D.

In this poster, machine learning techniques and models are presented that could be utilised to assess environmental degradability of a molecule before synthesis has even begun. The aim of this modelling is to produce a useful tool for medicinal chemists that can guide design choices, alongside the other computational tools utilised during the initial multiparameter optimisation processes in early stages of drug development.

By using publicly available biodegradation data obtained from the Organisation for Economic Co-operation and Development (OECD) Ready Biodegradability, Inherent Biodegradability and simulation tests, machine learning models were built in order to successfully classify the biodegradation potential of small molecules. The models achieved high balanced accuracy across both binary and multi-class classification tasks. From these models, structural insights could be extracted through the use of SHapley Additive exPlanations (SHAP) to suggest moieties that would enhance the biodegradability of a chemical. The applicability domains of these models were discussed, with exploration of chemical fingerprint similarity, nearest neighbors analysis and model confidence suggestion. Furthermore, counterfactuals were generated from the model to suggest structural modifications on the individual case, which may inform design. Although, due to the limitations of the training data available, the modifications generated may lack biological relevance, which is why this research is best utilised with the insight of an experienced medicinal chemist.

The proposed thinking could enable the collaboration between environmental risk assessors and medicinal design chemists in order to design non-persistent pharmaceuticals during the early stages of drug development, extending beyond current "rules of thumb" for degradation potential.

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7.10.P-Th330 Optimized Throughput in Aquatic Ecotoxicology: A Platform for Early-Phase Assessment of Agrochemicals

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To support the “Safety by Design” paradigm, the development of next-generation crop protection products relies on robust, high-quality ecotoxicological data generated during early phases of compound development. This comes with two challenges: the need to evaluate hundreds of molecules and their metabolites within a single chemical class, and the often very limited quantities of compounds available due to the time- and cost-intensive nature of de novo synthesis. While computational models offer scalable approaches for chemical hazard assessment, their reliability is fundamentally dependent on the quality and breadth of experimental data particularly for novel chemical classes outside established applicability domains. These constraints highlight the critical need to scale up ecotoxicological hazard assessments without compromising data quality or regulatory relevance.

To address these challenges, BAYER’s Early-Environmental Safety (eEnSa) Department has developed a high-throughput aquatic ecotoxicology screening platform that enables parallelized acute toxicity testing in miniaturized well-plate formats. The platform supports simultaneous testing of six regulatory-relevant aquatic species (*D. magna*, *C. riparius*, *Desmodesmus* sp., *Raphidocelis* sp., *L. gibba*, and *W. arrhiza*). Through parallelization and miniaturization, the amount of compound required for all six assays is reduced to as little as 1 mg or less, making comprehensive screening feasible with limited material within a short period of time. Automated liquid handling, dilution series preparation, and compound application ensure high reproducibility and standardization, while integrated image analysis algorithms enable objective and efficient endpoint quantification. Preliminary validation with sets of reference compounds demonstrated strong concordance with endpoints reported in the PPDB or the EPA’s ECOTOX Knowledgebase, confirming the reliability of the approaches.

This platform generates high-quality, comparable data to serve as a foundation for future predictive modeling and supports early identification and prioritization of safer crop protection candidates. Advancements in automated video tracking, image analysis, and data processing will further increase throughput and sensitivity, enabling earlier detection of sublethal and behavioral endpoints. This approach advances efficient, data-driven hazard assessment within the safety-by-design framework for plant protection product development.

Disclaimer/Disclosure:

I declare no personal conflict of interest. This research is funded by Bayer AG Division Crop Science.

7.10.P-Th331 Image processing and acquisition for the automated assessment of ecotoxicological effects

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For the development of new crop protection active ingredients, we at Bayer Crop Science pursue the "Safety by Design" approach. This requires conducting ecotoxicological environmental hazard assessments for hundreds of molecules at an early stage of research.

Classical evaluation methods cannot guarantee this throughput without significant personnel resources and also introduce subjective interpretation possibilities. Therefore, we strive for a high degree of automation in biological assays to optimize data acquisition and apply modern algorithms.

Our system automates data acquisition and processing, thus ensuring the necessary throughput. The frame rate can reach up to 70 frames per second (FPS), and with a 96-well multititer plate, we achieve a resolution close to full HD per well. These technical features enable precise analysis of various organisms using image and video recordings.

The developed software allows for standardized and repeatable analysis of the recordings. Multiple organisms in a single well can be segmented, counted, and tracked. The current state of development makes it possible to determine movement patterns, distance, speed, and length, for example, of *Daphnia* sp. as well as determining the size or pigmentation of *Wolffia* sp. Automated image recognition and tracking enable the reproducible detection of sublethal effects, increasing the throughput of biological assays. Furthermore, the archived image data allows for the continuous improvement of evaluation metrics using new algorithms, eliminating the need to

repeat experiments. In the long term, our approach aims to detect sublethal effects and develop models for predicting chronic ecotoxicological studies.

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I declare no conflict of interest.

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7.10.P-Th332 Safety aspects of novel fluorine-free coatings under the SSbD framework

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Concerns over the impacts of per- and polyfluoroalkyl substances (PFAS) on human health and the environment and resulting regulatory restrictions have created a pressing demand for benign alternatives within coating applications where PFAS are commonly used. 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 innovative PFAS-free coatings that are safer and more sustainable than current PFAS-based water- and oil-repellent coatings for use in the packaging, textile, and kitchenware sectors. To ensure that the novel coatings meet safety and sustainability criteria, TORNADO aligns with the European Union's Safe and Sustainable by Design (SSbD) framework. The innovation thus proceeds in parallel with an SSbD assessment. The safety assessment in line with the SSbD framework is addressed by the authors. As a first step, a comprehensive hazard assessment of the intrinsic properties of the chemical and materials involved in the synthesis of TORNADO coatings has been performed using a combination of scientific literature and in silico QSAR-based prediction tools. The assessment is continuously updated with new substances added to align with developments in coating synthesis. As a second step, a human health and environmental risk assessment of the manufacturing and processing steps within TORNADO was conducted. The assessment employed the Chemical Agency's Chemical Safety Assessment and Reporting (CHESAR) tool and its integrated models for human exposure and environmental fate, including the ECETOC Targeted Risk Assessment (TRA) and the European Union System for the Evaluation of Substances (EUSES). Since several chemicals involved in the processes are new or uncommon, leading to challenges in the collection of input data for risk assessment tools, a workflow for data collection was set up involving the use of both literature data and in silico predictions. Finally, the risk assessment of the use phase of TORNADO coatings is currently ongoing, in parallel with an evaluation of their chemical footprints throughout their life cycle using USEtox. At the same time, the broader environmental, social, and economic impacts are assessed by partners in the project, and a full SSbD assessment in line with the framework will be produced through a joint effort in early 2026.

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7.11.P Regulatory Implications of Digital (Precision) Agriculture: Environmental Risk Assessment in Time and Space

7.11.P-Mo437 Precision application of pesticides in agriculture: update of the European Precision Application Task Force (EUPAF) for field crops

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An update of the work of the European Precision Application Task Force (EUPAF) on precision pesticide applications in European field crops is proposed. This update outlines the task force's structure, approach, findings, and implications for regulatory risk assessment and sustainable agriculture. Established in June 2023, EUPAF comprises 127 experts from 60 organizations across 19 countries, aiming to integrate precision application technologies with regulatory frameworks for plant protection products. It is organized into five task groups addressing inventory, use descriptions, risk assessment, and case studies. The task force compiled a

detailed inventory of precision application techniques used in two-dimensional field crops, categorizing them into band, patch, and spot applications, with variable rates possible within each category. This inventory includes traditional and new technology sprayers and reports usage reduction percentages. TG2 developed standardized descriptions and dedicated language for Good Agricultural Practice (GAP) tables used in regulatory dossiers, based on existing EPPO and ISO definitions, and prepared a supporting handbook and glossary. Precision applications generally reduce exposure risks by lowering application volumes and targeting treatments, though factors like application location, resolution, and timing also influence exposure to crops and non-target organisms. Current risk assessments use realistic worst-case scenarios, but EUPAF proposes tailored assessments reflecting precision use benefits. The task force is working on refining exposure assessment methods by incorporating treated area and developing more complex models that integrate real-time parameters such as weather and geolocation to enable situation-specific risk assessments. Finally, data are collected to create case study documents illustrating precision use categories, serving as practical templates for regulatory contexts. Precision application technologies can contribute to reducing the environmental footprint of plant protection products and support sustainable agriculture, but they require evolving regulatory assessment models to quantify benefits and support decision-making. EUPAF aims to provide foundational scientific elements for this evolution.

7.11.P-Mo438 Ecotoxicological Implications of Precision Spray Technologies: Challenges and Opportunities

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As part of the UK Health and Safety Executive's initiative to develop a robust independent pesticides regulatory system for Great Britain, a report was commissioned to explore how precision spray methods might impact the ecotoxicological risk assessment of non-target organisms (NTOs) under the GB regulatory framework.

The aim of this report was to evaluate the implications of applying the current ecotoxicological risk assessment framework to precision spraying practices, specifically spatially variable rate applications, band, and spot spraying, and to identify knowledge gaps. Our analysis focused on key organism groups: birds and mammals, bees, non-target arthropods, soil organisms, and non-target plants.

Precision application techniques introduce spatial heterogeneity in pesticide distribution within fields and off-field habitats, creating opportunities for risk refinement, but also new challenges. For some birds and mammals, chronic risk can be refined if accounting for foraging patterns and habitat overlap, while acute risk cannot be refined. Although bee colonies may benefit from lower overall pesticide use, their high mobility limits the reduction of exposure for individuals, and spray drift remains a concern. Non-target arthropods (NTAs) and soil organisms show potential for recovery from untreated refugia, but population-level effects depend on species ecology and recolonisation dynamics. For non-target plants, untreated areas and buffer zones may provide meaningful mitigation, though drift-driven impacts persist.

Overall, precision spraying can reduce overall pesticide input and offers a pathway to align crop protection with biodiversity goals. However, current ecotoxicological frameworks assume uniform exposure and do not capture spatial variability or recovery potential. Integrating precision technologies into risk assessment will require new ecological data, refined exposure factors, and standardised approaches to account for species-specific traits and landscape-level effects. This work highlights the need for a paradigm shift in ecotoxicology, moving from uniform exposure assumptions toward dynamic, spatially explicit assessments that reflect modern agricultural practices.

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7.11.P-Mo439 Recent Developments in Spray Drift Modelling for Precision Applications

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Precision application of plant protection products (PPPs) is a promising way forward in pesticide application, since it has the potential to reduce the amount of PPPs applied and to reduce the exposure of different off-field areas and non-target organisms to PPPs. Precision application for crop protection could play a role in the exposure assessment of PPPs in the authorisation process. Basically, variable-rate and spot-spraying

applications can be distinguished. In a variable-rate application the whole field is treated while the dose is varied to meet the conditions of each location in the field. In a spot-spraying application for each in-field location the decision is made whether or not a (full dose) spray application is required. This study deals with the modelling of the downwind deposits of spray drift after a PPP precision application. With precision applications, the downwind deposits not only depend on the environmental conditions but also on the application technique, including the geometry and layout of the treated spots in the field. Recent model developments focused on establishing a spray drift metamodel that can account for varying environmental conditions and spray application conditions, including drift mitigating techniques. The model can be used for both variable-rate and spot-spraying applications. Downwind deposits of spray drift are evaluated at off-field evaluation strips, such as adjacent watercourses or non-target terrestrial areas. Downwind spray drift deposits depend on the location and distribution of the treated in-field areas and the dose rates applied. Wind direction is the major environmental parameter involved. Example computations indicate that drift reductions with precision applications can be higher or lower than the reduction based on reduced amount of PPP. The spray drift model can be used on a single-field scale but it also has potential to be used in exposure assessment on a regional or national scale. In this way, the model can help to assess exposure to spray drift in a regulatory context.

7.11.P-Mo440 SPOTMOD: Simulation of run-off exposure from precision application by mechanistic modelling with VFSSMOD

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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 input on the field. Localized, high-resolution chemical application reduces off-field drift and surface runoff to adjacent water bodies and terrestrial habitats compared to broadcast application. 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 scientific and regulatory context.

The untreated field area can act as a sink for dissolved and adsorbed pesticides before the surface runoff exits the field, thus leading to a reduction of pesticide runoff and erosion losses additional to the effect of reduced applied mass. A new 2D mechanistic modeling framework, SPOTMOD, has been developed that considers i) sources: pesticide runoff generation from treated spots in the field, ii) sinks: infiltration, sedimentation and pesticide trapping on downslope segments of the field, iii) variable vegetation conditions during the cropping period and their effects on infiltration and sedimentation, iv) variable spot size and treated area fraction, v) variable spatial application patterns ((quasi)-random and observed), vi) 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 (or a real infestation pattern is imported). 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-VFSSMOD 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 is also developed using machine learning. The SPOTMOD metamodel is expected to make a significant contribution to the establishment of field-specific risk assessment/management approaches.

7.11.P-Mo441 Digital Farming - opportunity for landscape-specific risk management

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The authorisation of plant protection products (PPPs) in the European Union ensures a high level of protection for humans and the environment in accordance with legal requirements (Regulation (EC) 1107/2009). PPPs may only be authorised if it can be ruled out that they have unacceptable effects on the environment.

PPPs may only be authorised if it can be ruled out that they have unacceptable effects on the environment. In accordance with the uniform principles (Regulation (EU) 546/2011) it must be observed whether this protection

target can be achieved by directions of use if risks have been identified in the risk assessment. These conditions of use include the risk mitigation measures specified in the authorisation of a plant protection product (see also Regulation (EU) 1107/2009 – 36 (3) 3. (...) appropriate conditions may be imposed with respect to the requirements referred to in Article 31(3) and (4) and other risk mitigation measures deriving from specific conditions of use.

The presentation will explore two key questions:

- What requirements must risk mitigation measures meet in order to be implemented?
- What opportunities does "digital farming" offer for further developing area-specific risk management?

For the drift entry path, an established model is used to determine entries into non-target areas, which is based on the drift values according to Ganzelmeier and Rautmann 1995 et al.. Depending on the spraying technique used, this model shows a fixed relationship between distance and expected concentration.

This makes it very well suited for area-specific risk management in a landscape context. The opportunities offered by digital farming, taking into account GIS data and a pesticide application manager (see also projects such as PAM, OPAL), are highlighted. Consideration is given to which of the aforementioned requirements can be positively influenced here.

Finally, the question is raised as to what development opportunities digital farming offers for establishing more advanced risk mitigation measures with a greater reduction effect, such as greater distances of 30 m to surface water bodies with the simultaneous use of drift-reducing technology.

This should contribute to the discussions on digital tools and models to include site-specific information into risk management and assessment, ensuring that current procedures are prepared for future challenges.

7.11.P-Mo442 Field specific risk management for plant protection products: a “digitalized” way forward

Gunnar Kahl¹, Andrew C. Chapple², Frank Scherr², Robin Sur² and Thomas G. Preuss², (1)knoell Germany GmbH, Germany, (2)Bayer Research & Development Services LLC, Germany

Smart farming technologies and usage of farm management information systems implementing detailed geospatial data are used more and more commonly in agriculture. The authorization approach of plant protection products in Europe does not currently make use of these advances. A 90th percentile protection goal is currently often established based on a few realistic worst-case scenarios. Within this process the products receive authorization and mitigation requirements on the product label, which usually cover all fields, no matter if the field is very vulnerable or not. This is a pragmatic approach that may lead to sufficient protection of most fields while at the same time other fields are accepted as being underprotected.

To overcome the limitations of the currently implemented generalized approach, a transformation of the current risk assessment scheme towards a digital driven field specific risk management is proposed in three phases, which would be introduced step by step. The risk assessment procedure on EU and member state level would remain in large parts as it is. All three phases make use of the availability of farm management information systems to distribute field specific restrictions and mitigation requirements. In phase 1 the mitigation requirements, based on standard regulatory scenarios (e.g. FOCUS), are transferred to the specific fields which present the closest similarities to the given scenario with respect to soil and climate conditions. In phase 2, field specific modelling is performed where the standard parameterization can be adapted to local conditions. In phase 3, geospatial data are used to derive field specific parameterizations for the exposure and effect models. In all phases, each field receives application restrictions and mitigation requirements depending on the local situation which the farmers can fulfil by combining different mitigation options from a mitigation toolbox. With a field specific risk management, the protection goal is respected, and the protection level can be increased by imposing additional mitigation or application restrictions to vulnerable fields. These vulnerable fields could now be identified and receive specific protection rather than being treated like all other fields. The proposed system would decrease both overprotection of non-vulnerable areas (allowing optimised pest control and resistance management) and underprotection of vulnerable areas (by inducing field specific risk management options).

7.11.P-Mo443 Innovating Environmental Risk Assessment for Precision Agriculture and Drone Application Technologies: Emerging UK Regulatory Considerations

Melissa Reed¹, Claire Glennie², Pamela Johnson², Amy Stewart² and Grant Stark², (1)Health and Safety Executive (HSE) - Chemical Regulation Division, United Kingdom, (2)Health and Safety Executive (HSE), United Kingdom

Approaches to authorising plant protection products (PPPs) need adapting to more accurately characterise the risks associated with the application of PPPs using new technologies. The UK is currently exploring how we can adapt existing approaches for precision agriculture and applications by drones.

Precision applications have the potential to minimise the amount of PPP needed to provide effective control giving environmental benefits. Minimising use has the potential to reduce environmental exposure to non-target organisms which could be considered in the risk assessment, but the level of this reduction is uncertain and will depend the ecology of the species of concern. This poster covers some of our experience with different types of precision applications and explores the extent to which the risk assessment can be refined based on current knowledge and where new data and / or tools will be required.

Application via drone has different advantages and risks. They can be beneficial when the area to be treated is inaccessible for example in railway cuttings and bridges providing a safer working environment for operators. However more information is needed on exposures from spray drift as this will differ from conventional equipment. It is also not known if there is potential for increased soil exposure as a result of rotor down draft pushing the PPP through the vegetation layer, or removing PPP deposited on leaf surface from previous spray passes. HSE is working with industry to ensure suitable data are generated to support the regulatory process.

7.11.P-Mo444 Precision Pesticide Application and the Risk to Soil Organisms

Emma Tilston, Alice Tagliati and James Garratt, Enviresearch Ltd., United Kingdom

As part of the HSE's work to develop a robust independent pesticides regulatory regime for Great Britain this desk study investigated the change in risk profile of soil-dwelling organisms, under conditions of precision application, compared with whole-field application. Precision application practices will result in heterogeneous exposure where exposure frequency, duration and intensity will vary in ways that will alter the actual exposure to which different individuals of a population may be exposed. This differs from the current approach which assumes uniform application across the whole field and where spatial and temporal variability are largely ignored, as are chemical and organismal traits that enable recolonisation.

The current EU risk assessment framework was evaluated for three scenarios: 1) variable rate application based on a pre-defined map, on a spatial scale of >10 m, 2) pre-defined band spraying over crop rows, on a spatial scale of ~1 m and 3) spot or patch spraying, on a spatial scale of <1 m.

Knowledge of four aspects of organism ecology were identified as being required for the evaluation of risk:

- 1) the extrapolation of laboratory toxicity endpoints to field conditions
- 2) the spatial distribution of soil organisms
- 3) the mechanisms and rates of dispersal (and hence recolonisation potential) of soil organisms
- 4) the impact of agricultural practices on the spatial distribution and dispersal of soil organisms

It was concluded that the continued use of the current approach to risk assessment would be conservative. Calculations based simply on reduced average application rates would not provide an adequate level of protection. For earthworms and micro-arthropods, recolonisation is a plausible refinement option for band and spot applications, but an unaffected population in the untreated area(s) would need to be demonstrated. For microorganisms, in the absence of soil disturbance (tillage) natural recolonisation is unlikely to be fast enough to lead to recovery within regulatory acceptable timescales, thus refinement based on recolonisation would not be appropriate.

Ideally, an expert working group should be formed to (re)define protection goals and to specify acceptable recolonisation endpoints for target taxa. New, bespoke studies on the spatial variation of soil physico-chemical properties, soil organism distributions and recolonisation timescales as appropriate endpoints for risk assessment are required to address knowledge gaps.

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7.12.A Solutions from Source to Sink: Preventing, Removing, and Managing Pollution Across the Plastic Continuum

7.12.A.T-01 Household Micro- and Nanoplastics: Sources, Determinants, and Opportunities for Intervention

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Human exposure to micro- and nanoplastics (MNPs) remains poorly characterized, particularly regarding factors that influence inhalation exposure indoors. We focused on women of reproductive age, as pregnancy and early life are critical windows of heightened vulnerability to environmental contaminants. We conducted an observational study among 105 women in the Netherlands. Passive indoor dust samples were collected in glass petri dishes at baseline and after 3 months. Pyrolysis–gas chromatography–mass spectrometry quantified MNP composition and mass. Questionnaires captured sociodemographic and household factors, and environmental data were linked to home addresses. Determinants of MNP deposition were examined with multivariable mixed-effects linear regression. We detected MNPs in all samples, reported as clusters related to the respective quantifiable base polymers and accordingly include copolymers on a proportional basis. Polyethylene terephthalate (C-PET), polystyrene (C-PS), and polypropylene (C-PP) were most commonly detected with deposition rates up to 27.69 µg/m²/day. C-PET accounted for 80% of total deposition, while the next most abundant polymers, C-PP and C-PE, contributed 6.4% and 6.3%, respectively. Repeated samples collected three months apart showed fair reliability (ICC = 0.44). Higher deposition was observed in multi-person vs single-person households, and during fall and winter vs summer, whereas more living space per person was associated with lower deposition. Determinants explained up to 46% of the variance in MNP deposition. This study is one of the first in-depth assessments of determinants of indoor MNP deposition. We identified several consistent factors influencing exposure, explaining a substantial proportion of variability, although specific indoor sources remain largely uncharacterized. We hypothesize that C-PET and C-PP originate from textile fibers, which were visually evident in the processed sample but not fully captured in the questionnaire. Evidence-based strategies for exposure mitigation remain limited. While deposited dust samples provide a reliable measure of household exposure, source identification requires more targeted approaches and questionnaires offer only limited information. These findings lay the groundwork for future studies to refine exposure assessment and develop actionable interventions, including design improvements, behavioral modifications, and household strategies to reduce exposure to indoor MNPs.

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7.12.A.T-02 Reducing Microplastics Emissions from Clothing and Footwear – A Dynamic Probabilistic Material Flow Analysis Study

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Microplastics from clothing and footwear contribute to the growing plastics pollution problem. Actions from consumers, industry and policy makers can reduce the emissions of microplastic. Information on effectiveness of specific mitigation measures should facilitate this. In this study, thirteen mitigation measures aimed towards reducing the microplastic emissions from clothing to the environment were analysed for their emission reduction potential. This was done using an existing modelling approach using material flow analysis (RIVM report 2024-0106), which for the first time included footwear.

Consumption of clothing is estimated to have released 290 ton (250 – 340 ton) microplastics and Footwear 140 ton (120 – 160 ton) of microplastics to the environment in the Netherlands for 2022. In Europe consumption and manufacturing of clothing is estimated to release 8100 ton (7100-9200 ton) and footwear 3100 ton (2600 – 3700 ton) of microplastics to the environment. This excludes emissions outside of Europe, e.g. due to manufacturing or end of life textile exports. Most clothing emissions enter the environment through outdoor air, mostly due to wear and tear of clothing. The second largest sink for clothing is surface water, which is caused by microplastic loss during washing or drying. For footwear, the largest environmental sink are soils because of shoe sole wear. Four of the thirteen mitigation measures aimed at microplastics loss had a reduction potential higher than 10% compared to the baseline in 2050, assuming a stringent degree of implementation. The mitigation measure with the highest reduction potential were (i) replacing synthetic materials with natural materials (ii) improving fabric quality to reduce microfiber release during wearing, washing and drying of garments; (iii) increasing the

lifetime of garments, combatting fast fashion and (iv) using more delicate washing regimes. A second scenario aimed at less stringent implementation, e.g. voluntary, showed largely the same effectivity of measures, but at a much lower reduction.

It is up to stakeholders in the textile value chain (e.g. policy makers, industry and consumers) to implement mitigation measures. Their cooperation is needed to this and realize the highest potential emission reduction. It is recommended to extend this analysis to include other environmental impacts and assess cost-effectivity of selected measures.

7.12.A.T-03 Advancing Plastic Release Modeling: Quantifying Macro- and Microplastic Emissions Across 30 European Countries and Incorporating Microplastic Size Distributions

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The global presence of plastics is a major environmental challenge of the 21st century. Addressing this issue requires detailed knowledge of environmental emissions. Material flow analysis (MFA) combined with release assessments enables quantification of plastic releases across the entire product life cycle. In this study, we developed a plastic release model based on MFA for the EU27, Switzerland, Norway, and the United Kingdom, comprehensively quantifying macro- and microplastic emissions of LDPE, HDPE, PP, PS, EPS, PVC, and PET for 2020. The model consists of ten aggregated product sectors, 45 product categories, 12 waste collection systems, and six recycling systems, accounting for 456 emission flows across all life stages. Geographical differences in emission factors, linked to consumer behaviors and waste management practices, were observed across countries.

For Europe as a whole, the emission factor was $0.187 \pm 0.018\%$ for macroplastics and $0.0404 \pm 0.0030\%$ for microplastics. The emission factors vary widely between countries. Microplastic emissions to soils range from $0.047 \pm 0.010\%$ in Latvia to $0.019 \pm 0.003\%$ in Belgium. Subsurface soil receives microplastics mainly from sewage leakage and construction pipe use, with Romania showing the highest emission factor ($0.008 \pm 0.003\%$). Macroplastic emission factors are higher than microplastic overall, with soil as the main receiving compartment, ranging from $0.52 \pm 0.18\%$ in Greece to $0.04 \pm 0.01\%$ in Lithuania.

We further incorporated microplastic size distributions into the model, including pellets, microbeads, fiber shedding, fiber production, weathering, and abrasion. We found that microplastic emissions in Europe primarily consists of particles in the 2–5 mm size range in terms of mass, largely resulting from agricultural plastic use and recycling. Peaks between 0.1 and 2 mm were also observed, resulting from various MP emission pathways. Varied size patterns for different polymers were observed, reflecting their different uses. This study provides a solid basis for identifying key plastic emission sources and guiding policies to mitigate plastic pollution in Europe. The size-specific results can be integrated into fate modeling and used to support risk assessment studies.

7.12.A.T-04 Removal of microplastic from sedimentation tanks by use of in-situ microflotation (ISM)

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The progressive sealing of urban areas has led to an increased surface runoff that transports pollutants, including microplastics (MP), to connected aquatic ecosystems. In this context, tire wear particles (TWP) and tire road wear particles (TRWP) are considered as the most abundant MP class. Due to the growing awareness of the toxicity of microplastics and associated pollutants of TRWP, the upstream treatment of surface runoff in sedimentation tanks prior to discharge into water bodies is becoming particularly important. In detail, the fine particulate fraction of total suspended solids $< 63 \mu\text{m}$ (TSS63) is contaminated with micropollutants, including MP. However, previous studies have shown that existing stormwater tanks frequently lack efficiencies for TSS63 (Sandoval et al., 2023). In general, the sedimentation efficiency of small particles is expected to be lower due to their high mobility.

In response to these issues, a monitoring with a pilot built using in situ-microflotation (ISM) has been developed and successfully transferred from the laboratory to the targeted use case. The results show that we were able to observe a greatly increased elimination of TSS63 and TWP for the ISM compared to the used sedimentation tank without ISM technology. The microflotation retrofit resulted in a substantial enhancement in performance, achieving TSS63 efficiencies ranging from 55 % to 80 % across a range of hydraulic loadings from 0.5 to 10 m/h, highlighting the independence from hydraulic loading. Regarding the removal of tire wear particles, microflotation achieved an average removal rate between 63 % and 81 % was achieved over a period of nine month. ISM is a scalable and modular solution for enhancing the removal of fine suspended solids and microplastics from urban stormwater. The capacity to be retrofitted into existing infrastructure or integrated into

new designs provides a considerable degree of flexibility for municipalities and utilities. In view of increasing anthropogenic pollution from urban surface runoff to water bodies, this is an important step forward for more sustainable water management and less environmental pollution.

Disclaimer/Disclosure:

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7.12.B Solutions from Source to Sink: Preventing, Removing, and Managing Pollution Across the Plastic Continuum

7.12.B.T-01 Towards Safer Food Packaging: Tackling Microplastics, Chemicals and Protecting Human Health

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Micro- and nanoplastics (MNPs) have been detected in various foods and beverages, leading to widespread human exposure. To prevent MNPs from entering foodstuffs, their sources must be identified. While current research focuses mainly on environmental contamination, the role of plastic food packaging and other food contact articles (FCAs), such as processing and transport equipment, as direct sources of MNPs in food remains largely overlooked, despite the extensive use of plastics in FCAs.

To address this gap, we systematically mapped the scientific evidence of MNPs in foodstuffs originating from the normal and intended use of FCAs, such as bottle opening, and published the FCMiNo dashboard. A limited subset of studies indicates that plastic FCAs can release MNPs into foodstuffs under intended or foreseeable conditions of use. In this first mapping, we included literature published before 2023. Currently, we update the systematic evidence map with studies published between 2024 and the end of 2025, and here we share first results.

Beyond MNPs, chemicals used in FCAs also pose safety challenges, as they include known hazardous and unknown substances that migrate into foodstuffs. Current regulatory requirements are insufficient to protect public health because they do not address mixture toxicity nor microplastics. We therefore propose a novel approach to establishing the safety of chemicals in finished FCAs. This approach places material inertness at the centre, as inert materials are also most suitable for implementing a circular economy.

To minimize the impacts of (plastic) food packaging and FCAs on humans and the environment, sustainability aspects beyond safety need consideration. The Understanding Packaging (UP) Scorecard is a science-based, freely available tool that implements the concept of inertness and can be used to select the safest and most sustainable foodware. It evaluates chemicals of concern and material inertness to score product safety. On top of that, the UP Scorecard considers climate change, water use, plastic pollution, sustainable sourcing, and recoverability to rate a product's overall sustainability. It demonstrates that reuse systems based on inert materials have the lowest safety and sustainability impacts.

7.12.B.T-02 Does environmental policy support the circularity and sustainability of fishing gear?

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Abandoned, lost, or discarded fishing gear (ALDFG) is a major source of marine plastic pollution, contributing to ecological, economic, and societal challenges across aquatic environments. Recent EU regulations, including the Single-Use Plastics (SUP) Directive with the Extended Producer Responsibility (EPR) schemes require new, circular approaches for managing fishing gear waste. A sustainable value chain for circular end-of-life (EoL) fishing-gear management faces numerous challenges. This work is a part of an EU-project SEARCULAR and it aims to develop and evaluate a port-based circular solution ("Blue Point") that enables efficient collection, sorting, and subsequent recycling of EoL fishing gear, while assessing its overall sustainability.

The work includes the identification, characterization and quantification of fishing gear waste along the Basque coast. Based on the data obtained, material simulation of future Blue Point operations was done, covering all stages: material reception, characterization, sorting, conditioning, and validation. Environmental impacts are quantified using LCA and they are combined with cost benefit analysis (CBA) to support the overall

sustainability assessment of the solution.

The study found that although the ecological impact of fishing-gear waste is widely recognised as a major issue in the marine environment, the actual waste volumes are relatively low. Integrating fishing-gear waste with other local waste streams can further improve economic sustainability. LCA results are still to be finalised, but they aim to verify the benefits of circular solutions compared to linear value chains.

The study provides a blueprint for port-based, circular management of fishing gear end-of-life (EoL) that is both feasible and environmentally preferable, offering a replicable model for sustainable EoL fishing-gear management. The integration of LCA, CBA and stakeholder perspectives ensures robust, actionable recommendations for policy and practice.

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7.12.B.T-03 Essentiality, Safety, and Sustainability: A Novel Policy Framework for Effective Plastics Production Reduction

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Plastic production is responsible for many impacts on the environment across the whole life cycle of the products, from the raw material extraction to the technologies used for remediation. Scientists, international organizations and policy makers agreed that the key to address the global plastics crisis is to reduce the production of plastic items. The aim of this study is to present a novel and objective policy framework to phase-out “non-essential”, “avoidable” or “unnecessary” plastics products drawing from the definitions of the Nordic Council and building on the essential-use concept proposed by the European Commission for implementation in the REACH Regulation, while expanding its application as a means of restricting the plastic production to only products that are safe, sustainable, and that have a use that is necessary for health and safety, or critical for the functioning of society.

Given the complexity and number of plastics materials, we propose that the framework should be applied to the whole plastic product or plastic component instead of single chemical substances contained in the material. The assessment would result in three potential outcomes: (1) the plastic product does not raise specific concern, its production and trade is allowed; (2) the plastic product is identified as non-essential, hence its production and trade would be phased-out without delay; (3) Plastics products which are identified as essential but unsafe or unsustainable are given a time-limited exemptions to develop a safer and more sustainable product, or system to ensure that essential needs are met.

In our view this framework offers an objective approach to facilitate consensus in negotiations while supporting the production and trade of essential, safer and more sustainable plastic products. We believe that this approach is material agnostic and should also be applied to plastic alternatives and non-plastic substitutes to prevent regrettable substitutions and outcomes.

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7.12.B.T-04 Designing Circular Plastics with Bauhaus Chemistry

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Less than 10% of global plastic is recycled, hindered by material complexity, economic constraints, exports, and informal waste streams. Plastic waste is projected to nearly triple to 1,014 million tons by 2060, dominated by short-lived applications such as packaging and textiles, while long-lived uses will create “locked-in” waste for decades. These trends underscore how far we are from achieving a circular economy for plastics.

A major barrier is the chemical diversity within plastics. Thousands of substances serve overlapping or non-essential functions, creating complexity that obstructs governance and recycling. Chemical simplification offers a pathway forward by reducing unnecessary diversity and prioritizing safer alternatives. Policies promoting

fewer, safer chemicals, combined with essential-use and safe-by-design principles, can guide innovation toward circularity.

We introduce the concept of Bauhaus Chemistry, inspired by Bauhaus design principles of simplicity and “truth to materials.” Bauhaus design (1919–1933) emphasized minimalism and functional use of materials, driving innovation and mass production with few industrial commodities such as steel and glass. Applying similar thinking to plastics means designing with fewer, high-quality materials for multiple functions, improving recyclability and reducing chemical complexity.

Commodity plastics—high-volume polymers like PET and LDPE—should be standardized for reuse and mechanical recycling within pure waste streams, supported by clear labeling and strict chemical controls. Examples include PET bottle schemes analogous to glass recycling systems. Non-commodity plastics, such as those in electronics, should be recognized as outside the circular economy, with a focus on safer chemistry rather than recyclability.

To accelerate this transition, we propose four principles of Bauhaus Chemistry:

Design more with less – minimize material and chemical diversity.

Inspiration through simplification – streamline complexity through design.

No waste, no waste exports – enable local recycling and reuse.

Localized production and material cycles – strengthen regional circularity.

Implementing these principles can reduce hazardous substances, enhance recyclability, and align plastics with circular economy objectives.

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7.12.P Solutions from Source to Sink: Preventing, Removing, and Managing Pollution Across the Plastic Continuum

7.12.P-Th333 The Drain is a Reactor: Assessing Interactions in the Wastewater System

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Polymers in Liquid Formulation (PLFs) are found in millions of everyday products, from soap to paint.

Wastewater treatment systems already receive an unprecedented mixture of products, and despite regulation, many inappropriate products enter wastewater directly or indirectly. When mixed with PLFs, these products can remain in wastewater systems, travel further, and interact with other products.

As global water stress increases, drives to reduce water use are rightly encouraged, reducing the volume of water entering wastewater systems and increasing product concentrations.

This combination of increasing concentration, PLF use and more products leads to increased potential for product mixing and a practically infinite array of end-of-life fates for any product. This makes assessment using conventional Life Cycle Assessment (LCA) impossible.

Our work aims to address this by developing product risk profiles based on interaction potential and impact. Work to date has understood the wastewater system, identified PLF-containing products, and considered those product family interactions which may cause most risk.

Understanding the wastewater system began with expert interviews. The second stage involved data collection on products entering the system, undertaken through workshops with individuals who identified themselves as engaged in climate change and environmental issues. Individuals were asked to consider a range of PLF-containing products, and to highlight whether they personally would dispose of them through the wastewater system, and whether they thought others would do so.

The final initial stage was the engagement of PLF experts from academia and industry. This group were shown a matrix of products and asked to rate potential interactions between product groups from benign to high risk. Results of this first phase highlight eleven product categories of note, including fertilisers and pharmaceutical products.

Work is now underway on modelling interactions between products. The goal of the work is to produce risk profiles for products, indicating the likelihood of environmentally damaging interactions in the wastewater

system. As a likely enabler of transport and interaction, PLFs will be the initial focus. Ultimately, the project will produce a model able to identify the highest risk interactions of a product in the wastewater system, and a series of a "must avoid" interactions which have the potential for high environmental impact if allowed to occur.

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7.12.P-Th334 Looking forward to the EU Urban Wastewater Treatment Directive (UWWTD): Development of a novel sampling and sample preparation approach for microplastic analysis

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The implementation of mandatory microplastic monitoring at wastewater treatment plants (WWTP) through the EU UWWTD represents a significant step towards tackling microplastic pollution. However, the implementation of the UWWTD is hindered by several microplastic monitoring frameworks are still not fully standardized with regard to sample preparation and sampling approaches. Looking into the future, it is reasonable to assume that the UWWTD will be linked to regulatory thresholds for microplastic concentrations. Therefore, uncertainties for assessing microplastic concentrations must be eliminated. Our study addressing these issues and demonstrate the effectiveness of a novel device called the MIPS (MicroPlastic Separation, patent pending) device, which can efficiently separate microplastics from wastewater matrices via microflotation. The separation mechanism is based on the hydrophobic interactions between finely dispersed air microbubbles (30-60 µm) and microplastics in an aqueous medium.

Regarding sample preparation approaches the presented method has advantages over others (e.g. density separation), as these are often combined with the use of highly concentrated, expensive, and environmentally harmful chemicals. Moreover, using the MIPS for sample preparation is fast (approx. 1 h), whereas settling times during density separation often exceed 24 h, preventing a timely assessment of microplastic concentrations during monitoring campaigns. The MIPS is also being tested as a sampling system capable of collecting high sample volumes and, hence, increasing the representativeness of collected samples. The system is capable for being scaled up far beyond a sampling volume of 1000 L. Our overall goal is to establish a tool that combines sampling and sample preparation in one step minimizing the need for further preparation steps. The newly developed MicroPlastic Separation device (MIPS, patent pending) clearly demonstrated its suitability to reduce some main components of WWTP efficiently while providing high and robust recoveries for microplastics of 68?10% for polystyrene particles. For the matrix removal, a quantitative reduction is obtained for model compounds (96,7% for cellulose and 99,9% for sand). This can improve time efficiency of monitoring campaigns and also adding strong sustainability features, as only water and air is used.

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7.12.P-Th335 Longitudinal and Vertical Distribution of Tire & Road Wear Particles and Microplastics in Contrasting Urban Bioswales

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Microplastics (MPs) and Tire and Road Wear Particles (TRWPs) are classified as emerging contaminants in urban stormwater, yet their distribution patterns within green infrastructures like Sustainable Urban Drainage Systems (SUDS) remains inadequately understood. A major knowledge gap persists regarding how the design, stormwater inflow dynamics, and hydraulic connectivity of SUDS, like bioswales, govern the retention and distribution patterns of MPs and TRWPs in soils. To address this, the study compares two structurally and hydrologically contrasting bioswales: in Nantes, France, located beside a very low traffic density road and functioning as the final unit in a multi-bioswale treatment chain, and one in New Jersey, USA, situated along a high traffic density roadway and receiving untreated first-flush stormwater through a concentrated inlet. Surface soils (0-5 cm) and cores (up to 45 cm) were collected across cross-sectional and longitudinal transects in both

bioswales and analyzed using pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) for TRWPs and polymeric MPs - polypropylene (PP), polystyrene (PS), and poly (methyl methacrylate) (PMMA). In the French bioswale, a decreasing contamination profile was observed from the inlet (4.7 mg/g) along the 11 m runoff flow path to the outlet (1.36 mg/g) of the bioswale. TRWP concentrations also decreased from road-adjacent soils toward the opposite slope. The USA bioswale showed sharp TRWP enrichment at the inlet (13 mg/g) and attenuation along the 48 m flow path to the outlet (1.76 mg/g), consistent with high traffic loading and direct first-flush inputs. TRWP in the transect from the road-adjacent points to the opposite side of the bioswale showed similar decreasing patterns as the French bioswale. PP, PS, and PMMA exhibited similar distribution and attenuation patterns in both sites as TRWPs but with lower concentration ranges. In core layers of both sites, decreasing concentrations were found with increasing soil depths. Through the distribution patterns obtained in the study, we highlight the efficiencies of the studied bioswales for TRWP and MP (PP, PS, PMMA) retention and that the structural configuration, inflow regime, and hydrologic pathways are significant controls on contaminant fate in bioswales. The study provides new field-based evidence for better understanding and optimizing SUDS design to enhance contaminant retention and reduce their downstream transport.

7.12.P-Th336 Polymer Identification and Plastic Additive Screening from Multiple Trap Fisheries

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Abandoned, lost and discarded fishing gear (ALDFG) is an abundant form of plastic pollution in water bodies worldwide. Previous research has focused on polymer composition of floating ALDFG such as nets, typically with end-of-life upcycling in mind. While 25 million pots and traps are lost internationally each year, there is little understanding about the plastic make up of traps and how they differ across fishery types. Moreover, fishing traps have relatively unique plastic components, such as vinyl coatings and rubber components, which are relatively understudied in the marine environment compared to buoyant plastics (polyethylene, polypropylene). In conjunction with a large-scale, multi-country derelict trap removal and data collection program (National TRAP program at the Virginia Institute of Marine Science), we have received samples of lost fishing traps for chemical characterization. These include American lobster, blue crab, spiny lobster, stone crab, and mangrove crab fisheries. Primary polymer analysis was completed with Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR). Pyrolysis - Gas Chromatography/Mass Spectrometry (Py-GC/MS) was used to confirm polymer identification and a non-targeted analysis of plastic associated chemicals (e.g., plastic additives such as UV-stabilizers) for a subset of derelict trap components. Our results show a variety of polymers within each trap type and significant differences between fisheries. We also identify a number of plastic constituents with potential toxicological concerns, and the most pragmatic options for end-of-life upcycling options. This research provides novel insight into the environmental ramifications of one of the most abundant but often forgotten forms of fishing debris.

7.12.P-Th337 Nanoplastic filtration using native wood membranes: A nature-based solution to water purification

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Nanoplastic (NP) contamination in water bodies is widespread. Removing NPs from water sources intended for human consumption requires efficient, environmentally friendly, and cost-effective methods. This study evaluates the use of native wood membranes to filter NPs from freshwater using a pressure-driven process. The performance of the filter was assessed for different tree species, membrane thicknesses and filtration cycles, by measuring NP concentration in the filtrate and NP deposition in the wood membranes. Retention efficiency varied widely between species. After a single filtration cycle, spruce, a common softwood species, reduced NP concentrations by approximately 90%, while poplar, a hardwood, only reduced NP concentrations by ~20%. These differences in retention efficiency are linked to microstructural differences between soft- and hardwood tissues. Increased membrane thickness and repeated filtration further enhanced NP retention in all conditions. This work demonstrates that native (i.e. without chemical modification) softwood membranes can be used in low-pressure filtration systems to remove NPs.

7.12.P-Th338 Exploring Dutch Rivers: Scaling of Dimensions and Concentrations of Plastics

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Plastic pollution is an environmental concern due to its widespread presence. While particle size and abundance are crucial for modelling ecological impacts, like ingestion, these factors are not yet well understood in rivers. Moreover, many studies focus on one size class of plastic particles. This study examined the relationships among size dimensions, size-concentration, and size-cumulative surface area of a wide size range of plastic particles collected with trawl nets from upstream and downstream sites in two Dutch rivers during 2023 and 2024. Measurements included the longest length, surface area, circumference, and particle count. Geometric means of particle concentrations and cumulative surface area concentrations were calculated for equally sized bins. Reduced major axis and ordinary least squares regressions were used to determine the slopes of bivariate relationships between size dimensions (length–surface area slopes: 1.46 – 1.83; length–circumference slopes: 1.02 – 1.07; circumference–surface area slopes: 1.30 – 1.76), size-particle concentration (slopes: 0.19 – 1.44), and size-cumulative surface area (slopes: 5.15 – 7.71). Significant positive relationships were found among size dimensions. A significant increase in particle concentration with increasing length was observed only at the length threshold, which was applied because microplastics were accidentally sampled. Overall, particle length was not a reliable predictor of particle concentration. However, cumulative surface area concentration increased significantly with length. This study is the first to explore the relationship between size dimensions and concentrations of plastic particles across a broad size range (micro to macro) in Dutch rivers, providing urgent baseline information for monitoring and impact assessment.

7.12.P-Th339 From Source to Sea: Identifying Macrolitter Sources for Sustainable Litter Management at the Yser River Mouth

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Aquatic litter pollution is a complex challenge and the sources of most items found in the environment remain poorly understood. Identifying these sources is essential for targeted decision-making, effective mitigation, and evaluating policy impact. In this study, we combined year-round riverine and coastal litter observations at the Yser River mouth in Nieuwpoort, Belgium, with the Matrix Scoring Technique based on Tudor & Williams (2004) for litter source identification, a method that links litter items to the human activities (i.e. fishing, agriculture) that release them. Our aims were to determine the major contributing sectors, compare riverine and beach litter composition, and assess seasonal differences across a riverbank, harbour, and beach location. Eight litter samples were collected at the Yser River mouth from autumn 2024 to autumn 2025, including all anthropogenic items > 2.5 cm. The samples were sorted into the EU standardized Joint List of Litter Categories (J-list), and the Matrix Scoring Technique was applied to assign different likelihood levels for each litter category to 54 pre-defined and standardized sources. Regional conditions were taken into account by using thematic maps and expert knowledge. Source contributions were calculated from item counts to produce the final likelihood matrix. The dominant sources of macrolitter along the Yser River mouth vary spatially, with tourism and households contributing across all sites but sewage outlets appearing only at the riverbank, harbour and boating activities only downstream, and marine inputs such as fishing most prominent at the beach. These patterns indicate a transition from mainly land-based recreational and residential sources upstream to a mix of urban-industrial, maritime, and tourism-related inputs toward the coast. This study shows that integrating standardized litter categorization with the Matrix Scoring Technique provides a robust, transparent, and scalable way to identify the human activities that generate macrolitter across aquatic environments. These insights can inform coastal management, targeted mitigation measures, user education, enforcement, and funding allocation. Our findings also highlight the need to develop practical interventions, including awareness campaigns addressing intentional littering. This work advances source-identification methods and contributes essential evidence for designing more effective, regionally tailored aquatic litter reduction strategies.

7.12.P-Th340 Microplastic Pollution and Ecotoxicological Impact of the surface waters and sediments of rivers collected in Japan

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Microplastic (MP) pollution is a major global concern increasingly recognized as a threat to human and planetary health. MPs enter the ocean through multiple pathways—including urban dust, textile washing, and fragmentation of marine plastic litter—but few studies have quantitatively assessed the ecological risks associated with different MP sources. Here, we conducted a preliminary ecological risk assessment of MPs in Japanese river surface waters and estuarine sediments, using measured concentrations and predicted no-effect

concentration (PNEC) ratios to inform mitigation strategies. For MPs in surface waters from 28 rivers, Q2 particle concentrations for the 1–5 mm, 0.3–1 mm, 0.1–0.3 mm, and 0.02–0.1 mm fractions were 2.3, 2.1, 1,300, and 4,400 MP/m³, respectively. Mass-based Q2 values were 0.087, 0.014, 0.015, and 0.010 mg/m³, indicating that particles 0.3–5 mm accounted for ~80% of total mass. Polyolefins (PP, PE) dominated all size classes (>60%), followed by PET (~20%), and fragments and fibers composed >90% of particles in the 1–5 mm and 0.3–1 mm fractions. For estuarine sediments collected from eight rivers, Q2 particle densities were 180, 98, 1,800, and 14,000 MP/kg dw, and mass concentrations were 2.4, 0.55, 0.011, and 0.022 mg/kg dw, with 0.3–5 mm particles contributing ~98% of total mass. Polyolefins accounted for ~30–80%, PET for <10–30%, and other polymers (e.g., PAN, PA) for 20–50%, with substantial variation across size classes. As in surface waters, fragments and fibers dominated the 1–5 mm and 0.3–1 mm fractions (~90%). Of particular note, even buoyant polyolefins (PP and PE) exhibited markedly higher composition ratios in sediments when particle sizes were <0.1 mm, demonstrating clear settling behavior. This suggests that miniaturization of particles may reduce their inherent buoyancy—potentially through biofilm formation—and thereby promote their transfer to bottom sediments. Such behavior implies the presence of high-concentration hotspots in coastal benthic environments. Under a worst-case scenario, concentrations of 0.02–0.1 mm MPs in estuarine sediments exceeded the PNEC at several sites. Given that polyolefins comprise 40–60% of domestic production and waste in Japan and continue to be released, settle, and accumulate in sediments, our findings indicate that polyolefin fragment MPs should be prioritized for detailed evaluation and development of release-reduction measures among MPs transported to the ocean via rivers.

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7.13 The FAIR Principles in Environmental Toxicology and Chemistry: Enabling Data-Driven Insights

7.13.T-01 FAIR and CREED: Partners in Better Risk Assessment?

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The FAIR Guiding Principles (FGP) for scientific data management and stewardship set out to improve the Findability, Accessibility, Interoperability, and Reuse of digital assets. To have scientifically robust risk assessments data needs to be findable, accessible and also should be reliable and relevant for its given purpose. The CREED approach (Criteria for Reporting and Evaluating Exposure Datasets) was developed to provide a systematic approach the consistent and transparent evaluation of the reliability and relevance of exposure data used in risk assessments. By combining the FGP and CREED it allows for scientifically robust, repeatable environmental risk assessments to be performed.

To perform environmental risk assessment for a substance, environmental exposure data, either modelled or measured, need to be available. Regulatory monitoring data can be obtained from many sources, including, online portals and direct requests to the data owners. However, the data use requirements vary significantly between jurisdictions, and data availability can vary from request-to-request from the same jurisdiction as well as between jurisdictions. The application of FAIR principles to methodological information related to the analysis of samples, for example method used and accreditation of the analysis, would also allow for more datasets to be fully assessed using CREED and provide a definitive conclusion following a published approach on the reliability and relevance of the data used in the risk assessment. When data is available, and it has been shown to be reliable and relevant for your given purposes, it is important that it is included in the risk assessment. By not using all of the data available, especially where the amount of high quality exposure data are a consideration, this can undermine the conclusions drawn and may require further expenditure of limited resources.

This presentation will show using recent experiences obtaining, using and reviewing European surface water monitoring data for regulatory sources and published risk assessments of how the FGP are applied inconsistently, how the reliability of these data can be questioned, and instances where data are available to

support and further assessment conclusions, but have not been utilised, and provide some recommendations of best practice and highlight issues associated with not making data available, using all available data and assessing data for reliability and relevance.

7.13.T-02 MassBank: An Open and FAIR Mass Spectral Data Resource

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MassBank was initiated in 2006 in Japan as the first open source, open-access and cross-vendor database for mass spectra. MassBank.EU was launched in 2011, which increased spectral contributions related to compounds of environmental and toxicological concern. Since then, MassBank has evolved into a cornerstone infrastructure for FAIR (Findable, Accessible, Interoperable, and Reusable) chemical data supporting small molecule-based research activities.

A redesigned web interface launched in 2025 provides improved accessibility and performance. All records are maintained under version control. Each release is archived as an independent dataset with a digital object identifier (DOI), ensuring long-term traceability. Automated validation has been setup to improve the data quality upon submission and to detect issues with existing records. The MassBank data format has been refined to include richer metadata, and the semantic data integrates spectral data within the wider ecosystem of linked open data. Furthermore, the SPectraL hASH (SPLASH) enables content-based access. With contributions from >50 partners worldwide, MassBank currently hosts 130,420 spectra of 20,206 compounds.

MassBank is fully interoperable with major global resources, including the MassBank of North America (MoNA), Global Natural Products Social Molecular Networking (GNPS), PubChem, the US EPA CompTox Dashboard, the NORMAN Database System (NDS), RforMassSpectrometry and more. These cross integrations have further enhanced the availability and reusability of spectra data across different platforms empowering many mass spectrometry-based investigations and supporting chemical identification, exposure assessment and risk analysis workflows. One example highlighting the impact of open spectral libraries is a recent Parkinson's disease exposomics pilot study by Talavera Andújar et al., where several of the significantly different compounds implicated in PD penetrance were annotated with Level 2a confidence using non-targeted LC-HRMS and all publicly available MS/MS libraries (including MassBank) integrated within MS-DIAL and MetFrag.

The growing number of resources using MassBank spectra and the increasing number of new contributions embracing the GitHub-based submission system exemplifies how coordinated FAIR-aligned infrastructure can foster cross-disciplinary collaboration and underpin data-driven environmental and human health risk assessments.

7.13.T-03 Using collaboration and iterative refinement to create customised, community-agreed FAIR (meta)data supporting systems: Data Management Plans

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The Partnership for the Assessment of Risks from Chemicals (PARC) project is committed to the development of a FAIR (Findable, Accessible, Interoperable, Reusable) data culture and the provision of technical methods, tools, and infrastructure for harmonisation to aid effective exchange of data and information generated and reused in different disciplines pertaining to chemical risk assessment (CRA). Due to the large size (~200 partners) and scale (7 years) of the PARC project, and various requirements for data management in the disciplines relevant to CRA, the research objectives in PARC are being met via a set of projects (smaller size

and fixed duration), each having a Data Management Plan (DMP) derived from and extending the overarching PARC DMP.

To address the data management needs of the individual projects, we introduced a customised machine-actionable DMP (ma-DMP) within the Data Stewardship Wizard (DSW) tool – a JSON-encoded mind map for data management and planning. The ~650 questions and sub-questions in the DSW DMP Knowledge Model (KM) are customisable to PARC's needs. Thus, a core part of the effort to create project specific ma-DMP was (i) developing the initial short list of questions by PARC's FAIR data experts, (ii) pilot testing the reduced question set with representative projects from toxicology domain, and (iii) revising (and refining) the initial list of questions after the pilot test. Additionally, domain-specific communities in PARC choose the FAIR Enabling Resources (FERs) and FAIR Supporting Resources (FSRs), e.g., Persistent identifier services, metadata schemas, controlled vocabularies, repositories, ontologies to develop the domain-specific reference FAIR Implementation Profiles (rFIPs) to help the automatic choices in the ma-DMP.

The user-friendliness of the PARC-project level DMP KM, reflected in factors, such as ease of use, questionnaire design, guidance, automation features like prefilling, and interoperability with other research information documents was appreciated during the pilot sessions and is expected to enrich the information contained in the DMPs. These ma-DMP can help to analyse the quality of information in the DMPs and detect convergence of FERs. In addition, the answers in the project-level DMPs will serve as an input for future updates of this overarching DMP and the answers can also feed back into updates of the domain-specific reference FIPs, promoting FAIR (meta)data in Chemical Risk Assessment.

7.13.T-04 eData: A tool and format for rapid extraction of exposure data from scientific articles

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Data collection, extraction and formatting is among the most tedious elements of conducting an environmental risk assessment (ERA). Maintaining oversight and quality control of heterogeneous datasets require expert knowledge and many hours of work. This makes it an obvious candidate for automation, as the potential to save time and money through a consistent, reproducible workflow is considerable. As more emphasis is placed on the Findable, Accessible, Interoperable and Reusable (FAIR) principles, need for such tools will increase.

We have developed a toolkit to address these issues in exposure data collection, consisting of a) an exposure data reporting format (DRF) in collaboration with Partnership for the Assessment of Risks from Chemicals (PARC), and b) an interactive data extraction app (eData) that walks users through the processing of data into this format.

Data are extracted from a source document using a Large Language Model (LLM). To reduce LLM randomness, we use a structured data extraction to constrain the output to the DRF and a controlled vocabulary. The user then validates and explores the data across a series of modules that cover relevant data and metadata, before entering measured values (generally chemical concentrations). This approach blends the advantages of LLM extraction (speed, efficiency) with human interpretation (accountability, reasoning ability) and speeds up extraction with minimal adverse effect on reproducibility. The tool also supports conducting a Criteria for Reporting and Evaluating Exposure Datasets (CREED) assessment using the extracted data, enabling rapid quantification of reliability and relevance of the paper's data.

Internally, we are testing eData tool on an Aggregate Exposure Pathway project assessing aquatic copper pollution in (near-)Arctic regions. However, we will recruit further testers to enhance its capability in related domains (terrestrial ecotoxicology, etc.) and overall user experience and design. In the long term, we will integrate this tool with a toxicity data toolkit (qData), with an eventual aim to providing an integrated platform for ERA.

This is to the authors' knowledge the most advanced exposure data extraction tool currently in development, and its degree of integration of human validation and LLM extraction is unique. Our toolkit will be a valuable addition to data-based ecotoxicology and contribute to the field's becoming FAIRer, to the benefit of scientists and stakeholders.

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7.13.T-05 Applying the FAIR Principles to Personal Exposure and Health Data

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The exposome is defined as the combined exposures that individuals encounter throughout life. The exposome greatly affects health, but the linkages are complex and poorly understood. Human biomonitoring surveys typically gather information about citizens' personal exposure and health, e.g. information about chemical occurrence in body fluids, chemical occurrence in the personal environment, lifestyle, and health effects. Integrating such data across different studies increases sample size and statistical power. Combining such data with other sources of information (e.g. geospatial data, socio-economic data) presents huge opportunities to clarify how the exposome affects health. However, barriers to such large-scale integration remain, including heterogeneous reporting formats, lack of structured information, and compliance with data protection legislation. This limits large-scale analyses and delays evidence-based policy decisions. The Findable, Accessible, Interoperable, and Reusable (FAIR) principles provide a useful framework to overcome these barriers.

The Personal Exposure and Health (PEH) data platform was developed to harmonize, validate, store, and securely share personal exposure and health data, in compliance with data protection legislation. As part of the EU Partnership for the Assessment of Risks from Chemicals PARC, the platform architecture is enhanced to better implement the FAIR principles. The information in the PEH Data Platform is structured based on community-developed data standards and vocabularies, which enables harmonization and integration of different studies. For data providers, the PEH data platform offers a clear and user-friendly workflow that enables data FAIRification and increases visibility. For data consumers, the PEH data platform provides on-request access to a large, structured, and curated collection of personal exposure and health data.

The FAIRification of the PEH Data Platform increases interoperability with other research domains: it will be integrated into the Environmental Exposure Assessment Research Infrastructure EIRENE. This will help underpin a better characterization of the human exposome. The structured nature of FAIR data opens opportunities for novel analytical approaches, such as large language models. Ultimately, FAIR data will enable cross-disciplinary research, strengthen evidence-based policy making, and drive innovation in public health and environmental protection.

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7.13.P The FAIR Principles in Environmental Toxicology and Chemistry: Enabling Data-Driven Insights

7.13.P-Mo445 qData – a web-based FAIRification workflow for (eco)toxicological dose(concentration)-response data

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Effect-based methods produce data across biological levels, from molecular to apical, which vary greatly in structure and format. This heterogeneity hinders integration into mechanistic frameworks like Adverse Outcome Pathways (AOPs) and limits their use in hazard and risk characterization. To address this, we developed a Data Reporting Format (DRF) and prototyped a web-based user interface (qData UI) to streamline data collection, validation, and analysis of quantitative dose–response data from (eco)toxicological studies.

qData is currently under internal and external testing using example datasets, including a case study on Diuron toxicity in *Chlamydomonas reinhardtii*. This dataset spans multiple biological levels and time points (6–72 h), capturing endpoints such as PSII inhibition, mitochondrial membrane potential, ATP content, and growth. The interface supports structured data and metadata entry, submission to different repositories (e.g., NIVA Risk Assessment database and Zenodo), benchmark dose (BMD) analysis for event sensitivity ranking, and future implementations will facilitate structural equation modeling (SEM) to quantify causal relationships and time-dependent dynamics across the AOP continuum. The UI and connected workflows will ultimately be integrated into the Source-to-Outcome Predictor (STOPredictor; <https://www.niva.no/source-to-outcome-predictor>), an UI linking environmental exposure assessment with hazard characterization for the risk assessment of single substances and mixtures.

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7.13.P-Mo446 Using Collaborative and Iterative Process to create a Community-agreed Semantic Framework unifying Materials Safety and Sustainability

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The EU's Safe and Sustainable by Design (SSbD) framework integrates functionality, safety and sustainability considerations into early innovation process for chemicals and (advanced) materials to facilitate life-cycle informed decision-making. Multiple disciplines converge in SSbD, including engineering and process design, toxicology, chemical, computational, environmental and social sciences. Each uses its own specialised lexicon, hence a shared semantic backbone for data exchange and comparison is imperative for the success of SSbD. Creating such a unified semantic framework means aligning concepts, identifiers (e.g., CAS, SMILES), existing relevant ontologies and metadata standards across disciplines to identify classes (sub-classes) and relationships, enabling establishment of an overarching upper-level ontology.

Within the PARC, PINK and INSIGHT projects, the necessary semantic framework is being built, through workshops with domain experts from relevant fields to reach a shared decision, to create rules that can be read (and verified) by machines. Interoperability is achieved by integrating relevant mid-and lower-level concepts under a mutually consistent top-level conceptualisation (upper-level ontology) that covers all SSbD criteria and the corresponding experimental and computational approaches. Available datasets are being annotated and mapped to the minimal / lightweight ontology schema (classes, properties, relations) and to instantiate ontology classes (i.e., create individuals). Feedback from domain experts is used to refine and extend the lightweight ontology schema in an iterative manner.

Existing features of upper-level ontologies (e.g., BFO or EMMO) and tools (e.g., OBO Foundry and the OntoCommons EcoSystem), and novel approaches based on Web Ontology Language and Semantic Web Rule Language are being used. The SSbD semantic framework enables new terms and their definitions to be proposed on-the-fly to constantly improve the semantic coverage, making these terms directly available by assigning provisional identifiers. Functionality is preserved via links and mappings, and automated semantic alignment and translation between ecosystems where necessary. The resulting consensus-driven ontologies are FAIR (Findable, Accessible, Interoperable, Reusable), ensuring efficient data sharing and optimum utilisation of resources. The unified semantic framework will facilitate consistent, interoperable, queryable decision support for SSbD activities.

7.13.P-Mo447 EIQuan: A Stacked Ensemble Learning-Based Predictor for Quantification of Nontarget Chemicals in GC-HRMS Analysis

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Nontarget analysis using gas chromatography coupled with electron ionization high-resolution mass spectrometry (GC-EI-HRMS) has become increasingly vital for identifying hundreds to thousands of compounds in complex environmental samples. Herein, we employed GC-EI-HRMS to profile chemicals in wastewaters from various treatment processes within an iron and steel corporation. To address the challenge of quantifying compounds without authentic standards, we developed a stacked ensemble learning model. A total of 910 compounds were identified in all wastewater samples, categorized into 19 subgroups mainly including heterocycles, esters, ketones, hydrocarbons, and alcohols with aliphatic, alicyclic, and aromatic structures. Considering these major categories, 278 reference standards were selected to develop a stacked ensemble learner. This model outperformed traditional semi-quantitative methods, with most of quantification errors falling within a 0.46-2.12-fold range (95% confidence interval). Total concentrations were quantified at 8.80×10^5 (influent) - 6.92×10^3 (effluent) $\mu\text{g/L}$ in coking wastewater and 309 (influent) -43.8 (effluent) $\mu\text{g/L}$ in mixed wastewater. Notably, 92.2% of compounds shared between the PubChem database and the NIST spectral library fall within the applicability domain of the model. To enhance accessibility, we developed a user-friendly predictor (EIQuan), an open-access predictor based on our quantification model, freely available on GitHub (<https://github.com/ECsPKU-LY/EIQuan.git>). This model provides an efficient tool for predicting response factors of environmental pollutants, significantly advancing the utility of complex GC-EI-HRMS data in environmental analysis.

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7.13.P-Mo448 Exposure Assessment of Consumer Products Using an Integrated Aggregate Exposure Pathway (AEP)-Adverse Outcome Pathway (AOP) Framework: Insights From Korean Market Data

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People are routinely exposed to a wide range of chemicals found in consumer products, including cleaning products, personal care items, toys and children's products, pesticides, and more. However, understanding the health effects of these chemicals remains a critical research gap, largely due to the lack of accessible product data, compounded by market globalization and limited transparency in product chemical composition due to the absence of stringent regulation and data reporting policies. To address this challenge, we utilized the Korean consumer product database, namely Ecolife, to conduct an exposure assessment of chemicals identified as high priority based on both exposure and hazard information. We accessed the products disclosed with complete chemical composition and harmonized the associated data using the vocabulary recommended by Organisation for Economic Co-operation and Development (OECD) and Government of Republic of Korea. Exposure data such as product information, chemical production volume and biomonitoring data were integrated with the hazard information from multiple sources to prioritize chemicals. Specifically, we focused on the chemicals cataloged in cleaning products and constructed their aggregate exposure pathways (AEPs) based on published evidence and monitoring data. To provide quantitative estimate, we performed exposure assessments for various conditions of use (COUs) and exposure routes using different exposure models to predict the chemical concentration at the target site. Subsequently, we applied the high-throughput toxicokinetics (HTTK) model to estimate the in vivo concentration present to initiate the biological response at the molecular level. This integrated approach establishes a framework for linking AEPs with the developed adverse outcome pathways (AOPs) for systemic toxicity of consumer product chemicals, thereby providing valuable insights to support regulatory decision-making and improve chemical management practices.

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7.13.P-Mo449 From Plastic Chemicals to Human Impact: An Architecture for AI Agents to Navigate Relational Databases

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The global crisis of plastic pollution is escalating, with mounting evidence linking chemical exposure from plastics to serious health and environmental risks. As concerns regarding plastic chemicals hazards continue to grow, development of tools that support linking their toxicological effects and identifying safer alternatives is needed. If chemical hazard data were easier to navigate and connect, researchers and policymakers could make timely, science-based decisions to restrict harmful chemicals. Currently, extracting and connecting meaningful information from chemical databases is time-consuming and requires specialized technical knowledge, resulting in promising ideas stalling. This hinders innovation in areas where speed and clarity are critical, such as unveiling plastic-related chemical hazards.

Generative AI offers the opportunity to use intuitive natural language to access structured data, such as complex chemical databases. Despite its potential to make scientific data more Findable, Accessible, Interoperable, and Reusable (FAIR), its applications in chemical and human-health databases are still underexplored.

To address this gap, we developed an AI Agent that interacts with ChEMBL—one of the largest chemical databases—using natural language. ChEMBL was selected for its high data quality and complexity, making it a suitable benchmark for our approach. The Agent consists of three core modules: (i) Relevance Validation, which

checks if the user's query aligns with ChEMBL's domain; (ii) Query Enhancement, which enriches the query with missing context and logical steps; and (iii) Table Retrieval, which uses Retrieval-Augmented Generation (RAG) to match the enhanced query with relevant database tables stored in a vector database, and then generates a corresponding SQL query. If the query fails, the Agent enters a feedback loop: the error message is appended to the original query and used to regenerate the SQL. This process is repeated up to three times, to optimize computation and time. If no valid query is produced, the user is notified. Results are displayed in a user-friendly table, which can be refined through natural language and downloaded as a CSV file. The tool can be used to assess bioactivity effects of common plastic chemicals.

This architecture can be extended to other chemical databases, such as our food contact chemical and human-health databases—connecting food contact chemicals usage with exposure and adverse health effects.

7.14.P Tracing Past Pollution With Unconventional Proxies: Legacy Footprints Guiding Today's Environmental Risk Decisions

7.14.P-Th341 Historical Foraminiferal Collections as a Resource for Monitoring Anthropogenic Impacts on Marine Environments

Laura Cotton¹, Carlos Edo¹, Nivedita Naveen², Emanuel Larsson² and Helena Filipsson², (1)Natural History Museum Denmark, Denmark, (2)Lund University, Sweden

Foraminifera within Natural History Collections (NHC) represent an underutilized yet valuable resource for tracking environmental changes and anthropogenic impacts on marine ecosystems. Foraminifera are protists with a (usually) calcareous shell frequently used in biomonitoring studies across marine environment. Historical foraminiferal samples, collected during marine expeditions from the late 1800s to mid-1900s, therefore offer crucial baseline data pre-dating significant human influence. These samples, often accompanied by oceanographic data, can fill important knowledge gaps regarding past biodiversity, species distribution, pollution levels and ecosystem health, but many remain unstudied within the collections of Natural History Museums around the world. While challenges such as limited accessibility, uncertain sample histories, and methodological inconsistencies exist, modern analytical approaches, including non-destructive imaging and advanced image analysis and machine learning, greatly enhance their usability.

Here we highlight the potential of foraminiferal NHC via several case studies representing ongoing work at the Natural History Museum Denmark, including: microplastic baselines in foraminiferal/sediment samples from the 1950-52 Galathea II expedition, morphological analysis of foraminifera from the G. Winther collection collected in 1872 and biodiversity studies of samples from the H. J. Hansen collection collected in the 1970s. We strongly advocate for the preservation, digitization, and strategic use of historical micropaleontological collections. In addition, we invite other researchers to consider the use of foraminiferal NHC in future research, emphasizing their potential for providing essential long-term ecological insights and supporting conservation and climate mitigation strategies.

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7.14.P-Th342 Foraminifera as historical archives of micro(nano)plastic contamination revealed by sub-micron O-PTIR spectroscopy

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Microplastics (MP) and nanoplastics (NP) are increasingly recognised as pervasive environmental contaminants, yet their long-term distribution in marine systems remains poorly constrained due to a lack of historical records. Agglutinated foraminifera, which incorporate external particles into their mineral-organic tests, represent a potentially valuable archive for reconstructing past contamination. This study aims to evaluate whether modern and historical foraminiferal specimens can retain small synthetic particles and thereby provide temporal insight into the deposition of microplastics and nanoplastics.

To address this, we apply a non-destructive workflow combining three-dimensional confocal fluorescence microscopy with sub-micron Optical Photothermal Infrared spectroscopy (O-PTIR). Confocal imaging is used to locate fluorescent micro-domains within the complex test structure, while O-PTIR analysis enables chemical

characterisation of these targeted regions at a spatial resolution of approximately 500 nm. Modern specimens from multiple regions are currently being analysed, and archived material from the Galathea II Danish expedition, collected before widespread plastic production, is being prepared to establish pre-impact baselines. Preliminary observations indicate that mineral autofluorescence can obscure natural signals, but dye-enhanced fluorescence improves the detection of regions of interest that may contain synthetic particles. Early results also confirm the feasibility of the integrated workflow for characterising fine-scale features within agglutinated tests. Historical samples will allow assessment of how synthetic particles entered these environments and how their incorporation has changed through time.

This study advances the development of high-resolution, non-destructive methods for detecting microplastics and nanoplastics below the approximate 20 µm limit of traditional techniques. The approach offers a new path to reconstruct the historical footprint of plastic contamination in marine sediments by using foraminifera as natural archives.

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7.14.P-Th343 Non-Destructive Portable X-Ray Fluorescence for Multi-Metal Assessment in Mussel Shells from Port Phillip Bay, Australia

Damien Nzabanita, Ecotoxicology Research Group, RMIT University, Australia

Trace metal contamination remains a key pressure on urbanised coastal systems, yet long-term records of exposure are often limited. Mussel shells provide a stable archive of environmental metal uptake, and portable X-ray fluorescence (portable XRF) offers a non-destructive approach to analysing these materials directly. Despite this potential, its application in marine biomonitoring has not been comprehensively evaluated. This study examined the performance of portable XRF for quantifying multiple trace metals in shells of *Mytilus galloprovincialis* collected from Port Phillip Bay in southeastern Australia. Ninety-nine shells were cleaned and scanned using portable XRF, and the same samples were subsequently digested and analysed by inductively coupled plasma mass spectrometry (ICP-MS) to establish reference concentrations.

ICP-MS data showed wide variation in metal concentrations across shells, reflecting a combination of natural background levels and localised anthropogenic inputs within this semi-enclosed bay. Portable XRF produced precise measurements and demonstrated strong correspondence with ICP-MS for several environmentally relevant metals, including barium, manganese, nickel and zinc. These relationships indicate that portable XRF is capable of resolving both spatial and biological variability in shell chemistry through rapid, non-destructive scans.

This work provides the first baseline dataset of shell metal concentrations for Port Phillip Bay and demonstrates that portable XRF is a practical tool for screening multiple trace metals in calcified biomonitoring materials. Its portability and minimal preparation requirements make it well-suited for large-scale surveys, retrospective analyses of archived shells and long-term environmental assessments. Integrating shell-based monitoring with portable XRF could substantially enhance the efficiency and spatial coverage of coastal contamination surveillance.

7.15.P Valuing Water Together: Transdisciplinary Collaboration to Achieve Actionable and Inclusive Water Quality!

7.15.P-Th344 Towards a Cleaner Meuse: Collaborative Strategies for Micropollutant Reduction in the River Basin

Ruud Steenbeek¹, Andre Bannink², Marjolein van Eerd³, Tineke Slootweg⁴ and Janneke Snijders⁵, (1)Dunea, Netherlands, (2)RIWA, Netherlands, (3)RWS, Netherlands, (4)HWL, Netherlands, (5)Aa en Maas, Netherlands

The Meuse River is a critical source for drinking water and biodiversity. Unfortunately, it is increasingly impacted by organic micropollutants such as pharmaceuticals, industrial chemicals, and substances of very high concern (SVHC). The partners within the Clean Meuse Water Chain initiative (“Schone Maaswaterketen” in Dutch) aim to enhance the water quality of the Meuse River and its catchment area. This advocates an integrated framework comprising four strategic pillars: Prevention, monitoring, collaboration and data integration. Prevention emphasizes source protection, revision of permits and responsible discharge management in collaboration with water authorities, drinking water companies, the government and industry, whereas

monitoring identifies critical compounds and establishes target concentrations to safeguard drinking water quality. A comprehensive monitoring program has been established to quantify target compounds across 30 sampling sites, applying advanced monitoring approaches. In addition, both direct and indirect discharges are being identified and linked to industrial facilities through a custom-developed database. The SMWK program illustrates how multi-stakeholder collaboration, innovative monitoring, and data-driven source control can accelerate progress toward cleaner water systems. Lessons learned will inform national and international policy. Continued efforts will focus on strengthening cross-border cooperation, and addressing emerging contaminants to underscore the necessity to ensure the security of drinking water resources.

7.15.P-Th345 Catchment Communities: An Interdisciplinary Approach for Innovative Citizen Science Water Quality Monitoring Tools

Nathan Maréchal, Chloe Martin, Charlotte Bouffeteau, Juan Esteban Quintero Marín, Fiona Regan, Fiona Murphy, Pdraig Murphy and Susan Hegarty, Dublin City University (DCU), Ireland

One of the primary challenges in water quality monitoring, is the significant amount of resources required to monitor extensive freshwater networks. In the case of the Irish river network, only 4% of all river bodies are actively monitored by the Environmental Protection Agency (EPA), the small order bodies being excluded due to a lack of resources. Therefore, there is a critical need to implement additional and reliable means of data collection. Doing so would allow for better support of policy-making, and help authorities reach the goals established in the European Union Water Framework Directive (WFD).

In recent years, citizen involvement has emerged as an effective way to gather reliable water quality data with an improved spatial resolution, complimenting traditional monitoring approaches in a desirable way. In this optic, the Catchment Communities project aims to design a framework for the development of citizen science (CS) water quality monitoring projects, easily scalable to a national scale. In order to address all aspects of the WFD, the project will cover hydromorphological and physico-chemical elements, while also focusing on the anthropological considerations of citizen science and water quality. This interdisciplinary approach is a key output of the project, as its presence in the framework will be essential for the successful creation of future citizen science projects.

This presentation focuses on the physico-chemical part of the project, demonstrating the application of existing and new CS tools for improved data gathering, sample collection and citizen engagement. The scope of development is driven by WFD needs, data gaps, EPA water quality reports and the potential to develop new robust tools that are reliable and citizen science ready.

Disclaimer/Disclosure:

This research is funded by the Environmental Protection Agency (EPA) of Ireland.

7.15.P-Th346 Participatory research of water bodies as a strong tool to manage environmental problems.

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The present investigation originated from concerns expressed by local social actors in the municipality of Vichadero, Rivera, Uruguay. Vichadero is a rural locality with very few nearby water bodies available for domestic, productive, and recreational use. The lack of information regarding the quality of these water sources had become a significant concern for residents of the village and surrounding areas. In response, local actors collectively demanded a scientific assessment of their water bodies.

Our research group conducted the first comprehensive study of the area using a participatory approach that incorporated local inhabitants' knowledge into the design of the monitoring strategy, including the selection of sampling sites, physicochemical parameters, and biological indicators. The objective was to identify the main environmental issues of the region through a socio-environmental diagnosis using participatory methodologies, while strengthening the collective capacity for knowledge production. This approach was complemented by a detailed analysis of physicochemical and biological parameters of surface water quality within the local micro-watershed.

The results revealed elevated concentrations of phosphorus in several water bodies, indicating potential

eutrophication risks. In addition, mutagenicity assays evidenced the presence of mutagenic compounds, raising concerns about possible impacts on environmental and human health.

These findings were presented to the community by the reference group that participated in the project, demonstrating local empowerment and an increased capacity to address their environmental challenges. Overall, this study provides evidence that similar participatory processes can successfully promote the engagement of local actors in monitoring and research initiatives linked to the environmental management of their territory.

7.15.P-Th347 Economic Valuation of Water for Better Policy Decisions

Thea Marcelia Sletten¹, Allan Provins² and Russell Drummond², (1)Economics for the Environment Consultancy (EFTEC), London, United Kingdom, (2)eftec, United Kingdom

Improving water quality requires evidence that captures the real benefits of reducing pressures on rivers, lakes and coastal waters. Yet much of the existing economic evidence is outdated or too narrowly focused to reflect today's challenges, including the growing importance of long-term chemical risks. This research addresses that gap by linking changes in waterbody condition to ecosystem-service outcomes using a pressure-to-benefit pathway. The valuation tasks focus on three clearly defined dimensions of condition: ecological status, suitability for contact recreation and long-term chemical risks such as those associated with PFAS, pharmaceuticals and microplastics.

The resulting evidence can be used to strengthen assessments for river-basin management planning, wastewater-treatment investment and chemicals policy by capturing benefits that have previously been difficult to quantify – particularly those linked to emerging or uncertain future risks. By combining ecological, recreational and chemical-risk dimensions within a single structure, the work offers a practical way to connect scientific understanding of pressures with the societal benefits of reducing them. This supports clearer prioritisation of actions and more coherent decision-making across sectors involved in protecting and improving the water environment.

Disclaimer/Disclosure:

The study is commissioned by Department for Environment, Food & Rural Affairs

7.15.P-Th348 Valuing micropollutants in LCA: are Waste Water Treatment Plants Good for the Environment?

Niels Jonkers¹ and Josje Brouwers², (1)Waternet, Netherlands, (2)KWR Water Research Institute, Netherlands

Dutch water authorities are increasingly using Life Cycle Assessment (LCA) as a decision making tool in water management projects such as dike improvement and wastewater treatment. In design variant studies, different options are evaluated on efficiency, costs and total environmental impact. Comparisons are made based on energy and chemicals consumption and the use of building materials. In these studies, the impact on water quality is often not specifically included, due to a lack of data or method availability. For the analysis of a wastewater treatment plant this could mean that only the environmental costs are taken into account, and not the environmental benefits.

Emissions of nutrients to water, such as nitrogen and phosphorus, could already be included in a relatively straightforward way. For emissions of micropollutants, this is a lot more complex. In the current project, we are taking on the challenge of quantifying the effects of micropollutants in LCA, with the evaluation of wastewater treatment techniques as a main application. The LCA impact assessment method most commonly used in the Netherlands by the construction sector and governments is EN15804 (with total scores expressed as an Environmental Cost Indicator, ECI or MKI). We aim to align with that method. The USEtox fate and effects model is used for deriving characterisation factors of micropollutants. Emission data is partly based on monitoring data and partly modeled.

In general this project hopes to contribute to an increasing awareness that water quality and in particular micropollutants should and could be taken more seriously when using decision making tools that consider total environmental impact, such as LCA tools.

7.15.P-Th349 Effect-Based Methods to Study the Occurrence and Fate of Antibiotics in Wastewater and Surface Water.

Jaimy de Schepper¹, Tjalf de Boer², Peter Cenijn³, Timo Hamers³, Thao Nguyen⁴ and Corine J. Houtman¹,

(1)Het Waterlaboratorium, Netherlands, (2)BioDetection Systems BV, Netherlands, (3)VU University Amsterdam (VU), Netherlands, (4)Stichting Waterproef, Netherlands

Risk assessment of antibiotics (ABs) in water typically relies on chemical target analysis of individual ABs, which may underestimate the total toxic pressure due to limited coverage of AB classes and their transformation products (TPs). This study evaluated three in vitro bioassays - the MicroGLO, the WaterSCAN and the E. coli FhuAT – to provide an integrated view on the occurrence and fate of ABs in water for water quality managers and drinking water companies. The MicroGLO and WaterSCAN were developed with five modules to distinguish between classes of ABs, whereas the E. coli FhuAT bioassay was developed as a general measure of AB activity.

Spiking experiments using ultrapure water and surface water (both spiked with AB and non-spiked) showed that these bioassays can successfully distinguish between water samples that contain ABs and those that do not.

Next, a field study was performed by using these bioassays on influent and effluent samples of three wastewater treatment plants, and five surface water bodies used for the production of drinking water. Chemical target analysis and targeted screening were performed in parallel to identify substances that might have contributed to activity observed in the bioassays and to assess whether the current chemical analysis provides a comprehensive view of ABs in the water cycle.

All three bioassays detected AB activity in wastewater, where the MicroGLO and E. coli FhuAT also detected AB activity in surface water. Effect-based trigger (EBT) values were exceeded in several wastewater effluent samples, indicating potential risk to the environment even after wastewater treatment. As such, water managers can use such bioassays as an early warning system for ecological risks of AB.

Comparison with chemical analysis revealed that bioassays often detected activity when target ABs were absent, and vice versa. This discrepancy between bioassay results and chemical analyses offers an opportunity for water authorities to revise monitoring strategies and incorporate effect-based methods into regulatory frameworks.

These findings demonstrate that bioassays can bring an added value to water managers and drinking water companies to study the occurrence and fate of ABs in wastewater and surface water. Their combined use with chemical analysis offers a more comprehensive insight in the occurrence and fate of ABs to support water quality monitoring and risk assessment.

Disclaimer/Disclosure:

This work was supported by two drinking water companies in the Netherlands.

7.15.P-Th350 Assessment of industrial wastewater treatment plant effluent quality using bioassays

Nessia Fausta¹, Marthe de Graaff¹, Astrid Fischer², Tineke Slootweg³ and Corine J. Houtman⁴, (1)Evides Industriewater, Netherlands, (2)Evides Waterbedrijf, Netherlands, (3)Water Laboratory, Netherlands, (4)Het Waterlaboratorium; Amsterdam Institute for Life and Environment (A-LIFE), VU University, Netherlands

Evides Industriewater operates several industrial wastewater treatment plants in the Netherlands. In line with the goals of the European Water Framework Directive in 2027, the company aims to deliver a leading effluent quality. The challenge lies in its tangible translation. Effect-based monitoring can help answer it. Therefore, EIW has applied bioassays to analyse the effluent from one treatment plant location. The objectives were to assess the effluent quality and identify the toxic compounds, with the ultimate aims to track down the sources to optimize the treatment process.

The selected location received the discharges from more than 60 industrial facilities. The assessment used a basic set of bioassays relevant to surface water quality: Cytotox-CALUX and Alivibrio Fischeri Bioluminesce (similar to Microtox®) for general toxicity, anti-AR- and ER α -CALUX for endocrine disruption, PAH- and PXR-CALUX (xenobiotic metabolism). Measured responses were benchmarked against effect-based trigger values for ecology. Exceedance of these trigger values gave directions to pinpoint the responsible compound groups and help investigate the sources.

Three samples were taken, one per month. Het Waterlaboratorium handled the pre-filtering, solid phase extraction and analysis for each assay. The analysis followed the Key Factor Toxicity method by STOWA. The

weight factor from the ratio between the unit and the respective trigger value determined the risk classification using colours: excellent (blue), good (green), medium (yellow), high risks (orange) and hazardous (red).

Activities indicative of general toxicity did not exceed the trigger values (blue and green), implying that no risks were expected. Risks of xenobiotic compounds could not be excluded, given the red colour for CALUX-PXR and CALUX-PAH, the latter likely caused by dioxins, PCBs and/or PAHs in the effluent. It is still unclear which specific compound(s) are responsible for these risks. Comparison with other types of surface water shows insights of the effluent quality and treatment performance.

The assessment indicates that the EIW's wastewater treatment plant performs comparably adequate to the drinking water intake points but requires further optimization to prevent the release of toxic effects into surface waters. The situation may vary due to changes in seasonal patterns and influent quality; additional samples during dry seasons and detailed influent quality analysis were recommended.

Disclaimer/Disclosure:

The comparison with a municipal wastewater treatment plant is under embargo. It can be published/presented after the authors receive approval from the related parties.

7.15.P-Th351 Integrated Approaches to Improve Chemical Water Quality in Surface and Drinking Water

Corine J. Houtman¹, Geert Corné Franken², Carla Van der Neut-Marchand², Yvonne van Oorschot² and Ruud J.C.A. Steen³, (1)Het Waterlaboratorium & Amsterdam Institute for Life and Environment (A-LIFE), VU University Amsterdam, Netherlands, (2)Het Waterlaboratorium, Netherlands, (3)Rijkswaterstaat West-Nederland Noord, Dutch Ministry of Infrastructure and Water Management, Netherlands

Surface waters used as sources for drinking water production are increasingly contaminated with chemicals of emerging concern, originating from wastewater effluents, agriculture, and industrial activities. Conventional monitoring based on targeted chemical analysis alone cannot capture the complexity of these mixtures or identify unknown compounds. This limits the ability of water authorities and regulators to assess risks and design effective measures.

To address this challenge, we applied a combined approach using a panel of in vitro bioassays and high-resolution targeted screening, complemented by multivariate statistics and effect-directed analysis. The methods were applied to waste water treatment plant (WWTP) effluent, and to surface water (SW), process water and drinking water (DW) of 4 DW companies in the Rhine and Meuse catchments. Bioassays revealed eight types of biological activity, including endocrine disruption and oxidative stress, some exceeding effect-based trigger values for ecological or human health risk. Targeted screening detected over 600 contaminants, with distinct patterns between the Rhine and the Meuse catchments: pharmaceuticals dominated in the river Rhine, while agricultural compounds and steroids characterized the river Meuse. Multivariate analysis clustered locations by contamination profiles and identified key compound groups driving water quality differences. Effect-directed analysis confirmed causative chemicals behind observed bioassay responses.

For water managers and policy makers, these findings provide actionable insights:

- Prioritization of measures: Focus on pharmaceuticals in Rhine and pesticides in Meuse catchments.
- Source control: Upgrade wastewater treatment and strengthen pesticide admission limits to meet Water Framework Directive goals.
- Risk-based monitoring: Integrate bioassays with chemical screening to detect unknown hazards and guide interventions.
- Strategic planning: Use clustering and correlation analyses to identify hotspots and shared emission sources, enabling targeted mitigation.

This integrated methodology advances water quality assessment beyond single-compound monitoring, offering regulators and drinking water companies a science-based framework to safeguard ecological health and drinking water safety.

Disclaimer/Disclosure:

This study was performed within the framework of the Joint Research Program of four Dutch dune water companies: Dunea, PWN, Waternet, and Evides (DPWE).

7.15.P-Th352 Seasonal Occurrence and Water Quality Hazards of Per- and Polyfluoroalkyl Substances in Estuarine Systems across a Gradient of Effluent Contributions to Base Flow

HyeongYoung Choi¹, Adam Wronski¹, Sara Kamanmalek², Jacelyn Rice-Boayue³, Jaylen Sims⁴, Kevin Stroski⁵, Chong Chen⁶, Alistair Boxall⁷, Kenneth M.Y. Leung⁶ and Bryan W. Brooks¹, (1)Baylor University, United States, (2)Howard University, United States, (3)North Carolina State University, United States, (4)Oak Ridge Institute for Science and Education (ORISE), United States, (5)University of Antwerp, Belgium, (6)City University Hong Kong, Hong Kong (Greater China), (7)University of York, United Kingdom

Global estuaries provide diverse benefits to human populations, biodiversity and ecosystem services. Similar to other coastal systems, Gulf of Mexico estuaries in Texas, USA experience diverse anthropogenic stress, including base flows that are influenced by upstream wastewater discharges. Given the limited global information for the occurrence and water quality hazards of per- and polyfluoroalkyl substances (PFAS) in estuaries, we employed a hydrological model to examine 14 Texas estuaries across gradients of annual precipitation (~60-140 cm/yr) and wastewater effluent contribution to base flows. Conventional grab and time-weighted composite samples over a 24-hour period were collected from these 14 estuaries during wetter (Spring) and drier (Fall) seasons of 2024, the hottest year in Texas since 1895. We quantitated surface water concentrations of 24 PFAS via isotope dilution LC-MS/MS. We employed a two-factor generalized linear model and identified sum perfluoroalkyl carboxylic acid (PFCA), long-chain PFAS, and precursors concentrations to significantly ($p < 0.05$) differ between seasons, with higher mean concentrations detected in spring. Concentrations of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) did not exceed United States Environmental Protection Agency water quality criteria for aquatic life during either season across the 14 estuaries examined here. However, PFOS levels in these study systems consistently exceeded European environmental quality standards and Australian and New Zealand guideline values during both seasons by <99%. These results highlight an urgent need to better understand PFAS sources, exposure scenarios, and water quality hazards in global estuarine systems across gradients of urbanization and climatic conditions.

7.15.P-Th353 Gradually Emerging Threats and Their Impact on Sustainable Drinking Water Security: Dutch Insights

Dominique Narain-Ford, RIVM, Netherlands

Although monitoring drinking water as a final product has traditionally been the standard for determining its safety and quality, experience over the past decades has shown that this approach can often be insufficient—particularly in the context of gradually emerging threats. Such threats may go undetected until they have already impacted drinking water quality or supply. This article provides an integrated overview of current and emerging threats to the drinking water sector, offering insights from the Netherlands based on an analysis of supply plans from ten drinking water companies. The study identifies five main developments impacting water security: climate change, anthropogenic substances (including PFAS), socioeconomic trends, geopolitical relations, and the energy transition. Each development introduces specific challenges, ranging from the deterioration of water sources and stricter health standards to growing population demands, supply chain vulnerabilities, and infrastructural constraints.

The findings reveal that the achievement of key policy objectives—ensuring good quality and sufficient drinking water sources, as well as the sustainable production and supply of drinking water—is under increasing pressure. Anthropogenic substances and climate change particularly threaten source quality and availability, while socioeconomic developments such as population growth and labor shortages jeopardize infrastructure and service reliability.

To address these challenges, the article highlights the need for cross-sectoral collaboration and structural improvements in permitting, supervision, and enforcement. Recommendations include harmonizing threat descriptions across supply plans, supporting data-driven prioritization of local bottlenecks, and increasing attention to public trust and communication. The study underscores the urgency of timely, coordinated measures to safeguard the future security and quality of Dutch drinking water supplies.

7.15.P-Th354 A Transdisciplinary Journey to Establish Safe Operating Spaces in European Rivers

Jennifer Laurent¹, Katarina Cetinic², Paulina Ramirez-Monsalve², Anna Sobek³, James S. Sinclair⁴, Daniela Cortés Guzmán⁴, Elmar Becker⁵, Milo Leon de Baat⁶, Dennis Trolle⁷, Anders Nielsen⁷, Iris Pit⁸, Peter Haase⁴, Ciaran Murray⁹, Susanne Schneider² and Jes Rasmussen⁹, (1)Stockholm University, Sweden, (2)Norwegian Institute for Water Research, Norway, (3)Environmental Science, Stockholm University, Sweden, (4)Senckenberg Research Institute and Natural History Museum Frankfurt, Germany, (5)University of Amsterdam, Netherlands, (6)Chemical Water Quality & Health, University of Amsterdam (UVA), Netherlands, (7)WaterITech, Denmark, (8)Deltares, Netherlands, (9)Aarhus University, Denmark

The management of freshwater ecosystems increasingly requires approaches that integrate ecological, social, and institutional dimensions, and that recognize how water quality and quantity are defined and valued in practice. Despite significant scientific advances in understanding the multiple pressures on aquatic biodiversity, a gap remains between knowledge production and its operational use in water governance. The EU Biodiversa+ DESTRESS project (DEciphering temporal trends and safe operating spaces for river biodiversity within the context of multiple STRESSors) addresses this challenge through a transdisciplinary framework linking ecology, hydrology, toxicology, and social sciences.

DESTRESS investigates how interactions between flow regimes, chemical contamination, and land use influence river biodiversity across Europe. Using long-term macroinvertebrate time series and environmental data, the project aims to identify biodiversity change points and determine Safe Operating Spaces intended to guide sustainable water management that balances water quality and quantity needs for both riverine biodiversity and human society. Beyond modelling, DESTRESS involves water managers and authorities to co-create actionable solutions that balance human water uses with biodiversity conservation.

The transdisciplinary journey of the project highlights the challenges and opportunities of connecting scientific, regulatory, and local knowledge. Through collaborative workshops, dialogues, and iterative feedback processes, scientists and practitioners jointly explore what “good water quality” means in diverse socio-ecological contexts and how hydrological and chemical stressors can be better captured in assessment and management practice. Integrating social sciences helps reveal tensions between ecological objectives, regulatory requirements, and human water needs, while fostering mutual understanding among disciplines and institutions.

Building on co-creation dynamics and stakeholder engagement, this work aims to identify pathways through which transdisciplinary collaboration could lead to a more inclusive, context-sensitive, and actionable water governance. The experience gained from DESTRESS suggests that collectively valuing water—scientifically, socially, and institutionally—is essential for implementing adaptive, equitable, and resilient strategies that protect aquatic biodiversity and support multi-functional freshwater ecosystems under multiple stressors.

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Track 8: Special Sessions

8.01 - Implementation of the European Commission Roadmap Towards Phasing Out Animal Testing for Chemical Safety Assessment: Advancing Endocrine Disruption and Chronic Fish Toxicity

8.01.T-01 3Rs for endocrine disruption - a stakeholder view (I)

Natalie Burden, NC3Rs, United Kingdom

8.01.T-02 3Rs for endocrine disruption - a stakeholder view (II)

Laurent L.-M. Lagadic, Bayer AG - Crop Science Division, Germany

8.01.T-03 3Rs for chronic fish toxicity - a stakeholder view (I)

Heike Laue, Givaudan Suisse SA, Switzerland

8.01.T-04 3Rs for chronic fish toxicity - a stakeholder view (II)

Kristin Schirmer, Environmental Toxicology, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Switzerland

8.01.T-05 Closing remarks and next steps: moving towards the long-term goal of phasing out animal testing

Christopher Faßbender¹ and Georg Streck², (1)PETA Science Consortium Int. E.V., Germany, (2)European Commission, Belgium

8.02 Integrating Landscape Perspectives in Environmental Risk Assessment: From Protection Goals To Practical Implementation

8.02.T-01 Landscape management in the current EU policies and regulations on pesticides and the vision of the EU Commission for the future

Sofie Hofkens, European Commission - DG Sante, Belgium

8.02.T-02 From Individuals to Landscapes: Scaling Mechanistic Individual-Based Models for Landscape Ecological Risk Assessment of Non-Target Organisms

Alice Johnston and Harriet Gold, Cranfield University, United Kingdom

Ecological responses to environmental stressors are characterised by emergent properties arising from interactions between mechanisms and processes operating at lower levels of biological organisation. Population responses of non-target organisms to plant protection products (PPPs), for example, cannot be inferred from toxicity measurements alone. Instead, population outcomes emerge from how individuals respond to PPP exposure in combination with ecological processes such as movement, reproduction and resource use across heterogeneous landscapes. These responses are further shaped by interacting stressors including agricultural management, habitat fragmentation and resource availability, which influence both exposure and population resilience. Predicting ecological consequences of environmental stressors therefore requires approaches capable of linking individual responses to population outcomes across space and time.

Mechanistic individual-based models (IBMs) provide a powerful framework for representing these cross-scale dynamics because they explicitly describe how physiology, behaviour and life-history processes interact with environmental conditions to determine survival, reproduction and dispersal. However, scaling such models to landscape extents relevant for ecological risk assessment remains challenging because of the persistent trade-off between ecological realism and computational tractability.

Here we present a framework for scaling spatially explicit mechanistic IBMs by identifying the spatial extent at which emergent population behaviour stabilises. The approach involves simulating IBMs across progressively larger spatial extents and quantifying how key population properties, including abundance, biomass and spatial structure, change with increasing extent. As model extent increases, the emergence of new behaviours diminishes, producing an area–emergence relationship from which a Critical Emergence Threshold can be identified. This threshold represents the smallest spatial extent at which emergent dynamics stabilise while computational costs continue to rise, defining the ‘Medawar Zone’, where ecological realism and tractability are jointly optimised.

This framework provides a pathway for integrating mechanistic IBMs with spatially explicit landscape simulations to support ecological risk assessment of non-target organisms by linking heterogeneous exposure and effects of PPPs and other landscape stressors to population-level outcomes.

8.02.T-03 Concepts, strengths and challenges of integrating landscape models into an environmental risk assessment for non-target terrestrial organisms

Pernille Thorbek, Apd/EE, BASF, United Kingdom

8.02.T-04 Plenary discussion

Melissa Reed¹, Sonja Braaker² and Bas Buddendorf³, (1)HSE, United Kingdom, (2)BASF, Germany, (3)Environmental Risk Assessment, Wageningen University and Research (WUR), Netherlands

Questions/dialogue from/with audience stimulated by chairs if needed by questions such as:

- what is our envisioned state for the landscape-level ERA over the next five years?
- how do we move from our current position to that desired state?
- what is a concrete first milestone we can set to begin that journey?

8.03 - Pull Up a Chair: Connecting Science, Society, and the Next Generation

8.04 Uniting the Silos: Learnings from Multi-stakeholder Collaborations to Advance Chemical Safety Assessments

8.04.T-01 Multi-Stakeholder Collaborations: Concepts and Strategies

Kevin Elliot, Michigan State University

8.04.T-02 Case study I (Regulatory body): : Cefic-ECHA collaboration – regulator’s view

Sampo Karkola, ECHA, Finland

8.04.T-03 Case study II (Environmental NGO): The case of mixture toxicity: What conditions are needed for a successful multi-stakeholder collaboration?

Flora Borchert, CHEM Trust, United Kingdom

8.04.T-04 Case study III (Academia): Use of evidence multi-stakeholder participation to minimize pollution of PFAS and PMT substances

Hans Peter H. Arp, Norwegian University of Science & Technology, Norway

8.04.T-05 Case study IV (Academia): Transparency as a basis to confront conflicts of interest and capture strategies in the assessment of chemicals

Gabriel Sigmund, EDGE, Wageningen University and Research (WUR), Wageningen, Netherlands

8.04.T-06 Case study V (Industry): Polymer Biodegradability network: A joint approach from the fundamentals of biodegradability to sustainable products

Andreas Künkel, Research Biopolymers, BASF SE, Germany

8.04.T-07 Case study VI (EU institution): The development of the EU Commission roadmap towards phasing out animal testing for chemical safety assessments – a collaborative approach

Georg Streck, European Commission - DG GROW, Belgium

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