

The INRAM project

Patrick Roose, Michiel Claessens*, Els Monteyne and Colin Janssen

In Belgium a 4 year project called INRAM (Integrated Risk Assessment and Monitoring of micropollutants in the Belgian coastal zone) is currently underway. The project is financed by the Belgian Federal Science Policy Office and aims to assess in an integrated approach the risks of micropollutants to Belgian coastal zone ecosystems and man. Passive sampling will be used as an innovative approach to measure contaminant pressure in the environment. During the project, a large group of hydrophobic organics will be measured at nine sampling points with passive samplers. Passive sampling will also be used as a tool for in lab toxicity tests at constant and environmentally relevant concentrations. Through the 'reverse' use of passive samplers toxicity assays will be performed with complex mixtures of contaminants.

**Contact author: Michiel Claessens: Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, J. Plateastraat 2, 2 9000 Ghent, Belgium.[tel.: 09 264 37 07, fax: 09 264 37 66 email: michiel.claessens@ugent.be]*

Patrick Roose, Management Unit of the North Sea Mathematical Models (MUMM/UGMM/BMM), 3 de en 23ste Linieregimentsplein B-8400 Oostende, Belgium. [tel: +32 59 242054, fax: +32 59 704935, email: p.roose@mumm.ac.be].

Els Monteyne, Management Unit of the North Sea Mathematical Models (MUMM/UGMM/BMM), 3 de en 23ste Linieregimentsplein B-8400 Oostende, Belgium. [tel: +32 59 242059, fax: +32 59 704935, email: e.monteyne@mumm.ac.be].

Colin Janssen: Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, J. Plateastraat 2, 2 9000 Ghent, Belgium.[tel.: 09 264 37 07, fax: 09 264 37 66 email: colin.janssen@ugent.be]

1. Subject and objectives

1.1. State of the science, strategic importance and problem formulation

Our seas and oceans are a fundamental component of our global ecosystem and as such we need to protect the health of our marine environments. Hence, it is crucial that we are able to (1) detect anthropogenically-induced changes in our seas, and (2) identify the reasons for such changes. It is only through developing scientific understanding of these processes that we can advise on necessary and appropriate remedial responses.

The marine environment receives inputs of hazardous substances through riverine (including harbours) inputs and direct discharges as well as by atmospheric deposition. As such, marine systems are the ultimate repository for a cocktail of persistent chemicals. This means that organisms living in these ecosystems are exposed to a range of substances which have the potential to cause metabolic disorders, an increase in disease prevalence and, potentially, population effects such as changes in growth, reproduction and survival potentially leading to community level changes.

Little is known about the transfer of these chemicals to and their effects on, the different components of coastal/marine ecosystems. This hampers an ecologically sound risk assessment and/or derivation of science-based environmental quality criteria for these compounds.

Despite extensive research efforts, the impact of most of these chemicals on marine ecosystem health remains unclear. Some of the **main reasons** for this are:

- (1) environmental monitoring of these chemicals is usually based on chemical measurements in water, and to a lesser extent in other matrices such as sediments and biota,
- (2) the assessment of potential effects of these compounds is mostly performed for a single chemical at a time and with a limited number of standard species (not necessarily ecologically relevant for the evaluated ecosystem) under laboratory conditions,
- (3) both in these laboratory test, and in the very rare field-experiments, the biomarkers used as evaluation endpoints mostly have an unknown relevance for predicting/evaluating ecosystem level effects,
- (4) measurements using field-collected (exposed) species using the same type of biomarkers suffer from the same short-coming.

1.2. Objectives

The present project will - based on the recognized expertise of the consortium and the experience gained during ENDIS-RISKS network research on the development of novel approaches to evaluate the presence, distribution, effects and risks of potential endocrine disrupting chemicals (EDs) in the Scheldt estuary - build upon the developed know-how to establish an enlarged (not only EDs, additional study areas and assessment endpoints) and more focussed characterization of the risks posed by micropollutants in the Belgian coastal zone.

The **overall aims of the present project** are to:

- (1) study the transfer and environmental concentrations of established priority compounds (cf. OSPAR, WFD and the UNECE lists) and emerging pollutants (e.g. pharmaceuticals) transfer via the three Belgian coastal harbours and the Scheldt, to coastal waters,
- (2) apply an unique combination of novel field and laboratory ecotoxicological and chemical techniques to establish both effects and food chain transfer of these chemicals,
- (3) establish the relationship between local occurrence of hazardous compounds, ecosystem health and potential human health effects, through the use of consumer organisms as test/monitoring species (e.g. commercial fish, crustacean and mollusc species), and;
- (4) develop and evaluate a framework and toolbox for monitoring the chemical anthropogenic pressures on coastal ecosystems and commercial marine products.

This research project, possibly combined with other research projects with which active cluster formation will be sought, **will**:

- (1) develop and validate (relevance assessment) a number of novel chemical, ecotoxicological and ecological tools to evaluate the presence and ecological impact of micropollutants present in Belgian coastal waters;
- (2) contribute to the knowledge and data - including development and validation of extended integrated risk assessment and monitoring procedures - required to fulfil international regulatory requirements (see above: WFD and EU Thematic Strategy on the Protection and Conservation of the Marine Environment) and/or commitments (see above: e.g. OSPAR) and,
- (3) demonstrate the use of the developed tools and procedures for the further development of a sustainable integrated coastal management system for the Belgian coast and its marine resources.

2. Methodology

2.1. Research strategy

To reach the envisaged research goals a combination of new, fundamental and applied science is required. This will entail:

- (1) exposure assessment: next to application of chemical analytical procedures previously established in the partners' laboratories (cf. ENDIS-RISKS network), the development and validation of new 'integrative' techniques (SPE) for detecting these chemicals and developing for compounds which have recently been identified as possible contaminants in marine environments;
- (2) effect and bioaccumulation assessment: next to the application of selected validated ecotoxicological tools established in the ENDIS-RISKS network, the development and/or validation and application of new laboratory and field assays (toxicity and bioaccumulation) with new (ecologically and commercially relevant) test organisms;
- (3) ecological monitoring: the development and assessment of ecological endpoints for assessing changes in ecosystem health. This will include new research into the in-field ecological performance of species used in the laboratory and field ecotoxicological evaluations in addition to 'conventional' monitoring and evaluation, through surveys, of ecological status of the Belgian coastal waters.
- (4) risk assessment: application of state of the art risk assessment techniques to assess the impact on the marine systems. Additionally through bioaccumulation measurements and biomagnification calculations the potential effects of the detected hazardous chemicals on two new protection goals: man (consumer of marine products) and resident tern populations will be evaluated.
- (5) management tools: development of integrated risk assessment procedures for the routine assessment of the impact of hazardous chemical present in Belgian coastal water on ecosystem health and man.

2.2. Research Tasks

2.2.1. Task 1: Exposure assessment: identification, quantification and geographical distribution of micropollutants in Belgian coastal waters

Sampling:

The occurrence of and the contribution of the selected priority chemicals released by the three main Belgian harbours to pollution along the Belgian coast will be – for the first time -

assessed by establishing (at least) two sampling stations in each harbour (Nieuwpoort, Oostende and Zeebrugge).

For each harbour, one sampling station will be situated in the inner harbour near industrial or boating activities (e.g. pleasure crafts) and one station near the harbour mouth. This will allow to assess the presence of pollutant gradients. One additional monitoring will be located in the Sluice dock of Oostende as this enclosed, shallow lagoon is used for aquaculture activities (oyster and mussel culture). The lagoon is supplied with water from the inner harbour of Oostende.

To assess the influence of the Scheldt on Belgian coastal water quality, two stations within the estuary are selected: i.e. one near the city of Antwerp (Endis-Risks station No. 7) and one near the mouth of estuary (Endis-Risks station No. 1, Vlissingen).

Next to these 9 harbour/river stations an additional 9 sampling locations along the Belgian coast will be selected. For each harbour, two will be located in-shore and radiating from the harbour mouth (contaminant gradient). The remaining 3 stations will be located at least 5 kilometres from the coast, and will serve a 'controls' to evaluate average offshore concentrations of the selected contaminants. To ensure the use of existing information on chemical occurrence and ecological quality of resident communities, the sampling stations (outside harbours) will be, as much as possible, situated at locations which have been and are currently monitored in the context of existing programmes.

During the first year (exploratory phase) all sampling locations will be sampled twice a year (summer and winter). This will allow to assess a possible temporal effect at the different locations and enable us to further refine the inner harbour locations and the final selection of chemicals to be monitored (possible site- and time-specific differences). From project year 2 through year 4, one sampling campaign per year is envisaged. At each sampling time, sediment, water and biota (see below) will be collected. Sampling and sample preservation will be done according to the protocols established by the research consortium during the ENDIS-RISKS project. These procedures adhere to the recommendations of international organisations such as OSPAR. Sampling will be performed with the available research ships (e.g. Belgica and Zeeleeuw); for inner harbour and inshore samples a rigid hull inflatable vessel (Zeekat) will be used. Next to the collection of samples for chemical or biological analyses, temperature, salinity, conductivity, dissolved oxygen, pH of the sample will also be measured during sampling.

Table 1: List of Organic Micropollutants (OMP) which will be analyzed in this project.	
(Groups of) substances	Analytical methods available
<i>'Old' organochlorines</i>	
Chlordane	GC-MS
DDTs	GC-MS
Dieldrin	GC-MS
Endosulphan	GC-MS, LC-MS
Endrin	GC-MS
Heptachlor	GC-MS
Hexachlorobenzene	GC-MS
HCHs (<i>e.g.</i> lindane)	GC-MS
Metoxychlor	GC-MS, LC-MS
PCBs	GC-MS, GC-ECD
<i>'New' pesticides</i>	
Alachlor	GC-MS, LC-MS
Atrazine	GC-MS, LC-MS
Chlorfenvinphos	GC-MS, LC-MS
Chlorpyrifos	GC-MS, LC-MS
Dicofol	GC-MS, LC-MS
Diuron	LC-MS
Isoproturon	LC-MS
Simazine	GC-MS, LC-MS
Trifluralin	GC-MS, LC-MS
PAHs	
Priority set and/or individual PAHs	GC-MS, LC-FLU or LC-MS
<i>'New' organohalogens</i>	
BFRs: PBDEs, HBCD, TBBP-A	GC-MS/LC-MS
Hexachlorobutadiene	GC-MS
Pentachlorobenzene	GC-MS
Endocrine disruptors	
NP/NPEOs and related substances	LC-MS
Octylphenol	LC-MS
Dibutyl and diethylhexyl phthalate	GC-MS
PFOS	LC-MS

Chemical analysis:

The chemical compounds that will be analyzed and the methods available to the research consortium are given in Table 1. This selection is based on our experience on the analyses and occurrence of these compounds during the ENDIS-RISKS projects and on the very recent, excellent review of Roose and Brinkman (2005) who based their recommendations and selection on the EU WFD list. However, in contrast to the conclusions of the WFD, some additional OSPAR priority substances and the 'old' organochlorines are still included, partly because they are on the UNEP-POP list but also because of their environmental relevance. Next to the compounds mentioned in Table 1, the four metals (Hg, Cd, Pb and As) on the WFD priority list, tributyltin and some selected pharmaceuticals will also be analyzed. Possible candidates for this latter group are: painkillers such as ibuprofen and paracetamol; antibacterial compounds such as amoxicillin and erythromycin, β -Blockers like e.g. actononol and metoprolol; antiepileptics such as carbamazepine and felbamate; antihistamine like cetirizine and lipid regulators e.g. bezafibrate. These and other pharmaceuticals have been found in fresh and marine waters, and it has recently been shown that even in small quantities, some of these compounds have the potential to cause harm to aquatic life (Bound and Vouvolis, 2005). The final selection of pharmaceutical compounds has not been made and will be based on a in-depth literature analysis and information on their use in Belgium. It is anticipated that representatives of at least 5 classes of pharmaceuticals will be measured in this project. Analytical methods for the quantification of these compounds in seawater will be developed based on available literature and the experience of one of the project partners with the analysis of veterinary products.

Novel approach: Passive sampling to measure concentrations of hydrophobic organic micropollutants in water

Next to the above mentioned 'conventional' sampling and analytical techniques; this project will further develop and evaluate the use of passive samplers in the context of marine monitoring and ecosystem health assessment. This novel approach is central to several of the innovations envisaged to result from this project. Passive samplers will be used in the context of (a) chemical analysis; (b) as a surrogate to assess bioavailability and bioaccumulation (c) as a contaminant source for dedicated ecotoxicological (laboratory) assays.

(a) Concentrations of hydrophobic micropollutants in the water phase, such as polychlorinated biphenyls (PCBs) are often very low (< ppt level) due to their low water solubility. For this reason these hydrophobic chemicals are difficult to detect with current classical measurement techniques and often the concentrations are below the analytical

detection limits. Passive sampling provides a powerful tool to measure these hydrophobic contaminants, because of their affinity for these compounds. The uptake of hydrophobic contaminants by passive samplers is driven by a diffusive process. After collection of the samplers, analysis of these samplers is a matter of routine analysis. Back calculation to the waterphase concentration is possible by using uptake kinetics and equilibrium constants. The uptake of the compounds is controlled by the – prior to deployment - added performance references compounds (PRCs) (Huckins *et al.*, 2002; Booij *et al.*, 2002). We will deploy passive samplers at each sampling stations, collect and replace them at each sampling time and – through chemical analysis - evaluate their use for obtaining a complete overview of all contaminants present at the site.

(b) Assessment of the bioaccumulation potential of hydrophobic micropollutants is – in the context of monitoring - mostly performed by collection and analysis of biota living in the contaminated environment. As this is very time-consuming and expensive, passive sampling has been proposed as surrogate to mimic bioaccumulation of hydrophobic organic substances in aquatic organisms (van der Oost *et al.*, 2003; ICES, 2005). Recent results have indeed shown freely dissolved contaminants concentrations are related to the bioconcentration of these organic contaminants in mussels (Folsik *et al.*, 2002; Smedes and Koning, 2005) and other aquatic organisms (van der Oost *et al.*, 2003; Verweij *et al.*, 2004). A good correlation of the hydrophobic contaminant concentration between the passive sampler and the mussel was observed. It is suggested that passive samplers can therefore be used to predict concentrations of these contaminants in both water and mussels. Again, we will deploy passive samplers at each of the sampling stations and evaluate their use for obtaining a accurate estimates of bioaccumulation by comparing passive sampler results to body burdens observed in locally collected biota (benthic and sessile pelagic organisms).

(c) Conventional ecotoxicity (laboratory) studies are mostly performed by exposing standard test organisms (often not representative of resident species) to various concentrations of a single chemical compounds. This clearly does not reflect *in situ* conditions where the organisms are exposed to a large number of contaminants simultaneously (mixture toxicity). A novel application of the passive sampler technique will be studied in the project. Contaminants collected by these devices will be used as an exposure source in laboratory assays with the selected species (see 2.2.2.). The concentrations of the components of the contaminant mixture released from the samplers into the bio-assay vessels will reflect *in situ* exposure concentrations and will be constant in time. As such relevant lab to field (*in situ* tests, see 2.2.2) comparisons and extrapolations will be feasible.

Table 2: Test endpoints, types and relevance of the model species to be used in the laboratory and in situ (field) ecotoxicological assessments.		
Species	Test type	Relevance of species
<i>Sediment compartment</i>		
<i>Corophium volutator</i>	Whole sediment assay with field collected sediment (harbours and inshore sites only)	Model benthic species, transfer from benthos to higher trophic levels
<i>Water compartment</i>		
<i>Saccharomyces cerevisiae</i>	Yeast Estrogen Screen (YES) to detect presence of endocrine disrupting (ED) chemicals	No ecological relevance, but – based on the results of the Endis-Risks project – sensitive to detect the presence of ED compounds
<i>Phaeodactylum spec.</i>	Laboratory assays using realistic contaminant mixture from passive sampler and measurement of population growth rate	Marine micro-algae, primary producer, bases of food web.
<i>Mytilus edulis</i> (or oyster) larvae	Laboratory assays using realistic contaminant mixture from passive sampler and measurement of larval development In situ field (cages) exposure at sampling sites (harbours and inshore only), same endpoint	Abundant primary consumer, sessile benthic filter feeder; prey for various species including birds and man
<i>Mytilus edulis</i> - adults	Laboratory assays using realistic contaminant mixture from passive sampler and measurement of larval development In situ field (cages) exposure at sampling sites (harbours and inshore only) and subsequent measurement of Scope for Growth and Cellular Energy allocation in the laboratory.	Abundant primary consumer, sessile benthic filter feeder, prey for various species including birds and man
<i>Neomysis integer</i> (as model <i>Crangon crangon</i> ; the use of <i>Crangon crangon</i> will also be explored)	Laboratory assays using realistic contaminant mixture from passive sampler and measurement of invertebrate specific ED endpoints developed in ENDIS-RISKS. In situ field (cages) exposure at sampling sites (harbours and inshore only), same endpoints	Abundant epibenthic species, prey for various fish species. Crangon: important consumer product.
<i>Limanda limada</i> (or <i>Pleuronectes platessa</i>)	Laboratory, short term (2-4 day) assays using field collected juvenile dab; assessment of lysosomal stability (and possibly CYP1A) after exposure to realistic contaminant mixture from passive sampler Assessment of lysosomal stability (and possibly CYP1A) of dab collected at sampling sites	Ecologically important secondary consumer. Important commercial fish species, link to human food chain

2.2.2. Task 2: Effects and bioaccumulation assessment: linking multi-contaminant exposure to ecologically relevant effects on selected resident marine models species

Test organisms:

Based on their known occurrence, their ecological importance and, for some species, their importance for potential transfer of the contaminants to the human or avian food chain, laboratory and field ecotoxicological studies will be performed with the species presented in Table 2.

Test approaches: laboratory and *in situ* exposures

As can be concluded from Table 2, we propose to use a unique ecotoxicological approach combining novel assays and test endpoints (with demonstrated relevance for organism level effects and ecosystem health) with a new exposure system (reflecting environmentally relevant concentration of the contaminant mixtures). Additionally, attempts will be made to explain the observed effects (1) single compounds testing with selected and identified compounds occurring in the mixtures; (2) using relevant existing literature and (3) through the development of predictive Quantitative Structure Activity Relationships by combining effects, water chemistry and body burden data collected during the experiments. This is the first time that this type of approach will be developed and assessed in an attempt to improve our understanding of the relative toxicities of environmental contaminants present in marine surface waters.

- Laboratory assays: studies with contaminant mixtures originating from passive samplers

Passive samplers will be collected from the sampling stations (cf. 2.2.1) and deployed in uncontaminated control medium. The adsorbed contaminants will be allowed to desorb from the samplers resulting, after reaching equilibrium, in a realistic (for the field situation) mixture of the compounds. An aliquot of this solution will be used for complete chemical analysis while the remaining volume will be used for ecotoxicity testing with the following species/assays (see Table 2): the YES, the *Phaeodactylum* spec. 72h growth inhibition assay, the 48h fertilization and larval development assay with *Mytilus* or oyster, the 48h to 96h Scope for Growth assay with *Mytilus* adults, long-term exposures with *Neomysis*, and, 48-96 h assays with field collected *Limanda* assessing lysosomal stability in liver tissue.

This battery of tests will in an exploratory phase be used for evaluating 10 samples, after which the sensitivity, reproducibility, and general performance of the assays will be evaluated

and compared. Based on these results, a final battery of 4 assays will be selected to be used for the remainder of the project and for all samples.

Based on the results of the ecotoxicity tests with mussels and those of the chemical analysis it is envisaged to attempt identification some of the causes of the effects through quantitative toxicological interpretation (QTI) of contaminant concentrations in the tissues. The QTI is based on established Quantitative Structure-Activity Relationships (QSARs) and cause-effect relationships (i.e. between concentration of contaminants in mussel tissues and the SFG response; for details see Widdows & Donkin, 1992; Widdows et al., 1995).

Results from measured body burdens and toxicity tests may allow to establish similar QSARs and resulting QTI assessment for some of the other species. Validation of the established relationship for selected test species will be pursued through the performance of dedicated experiment with single compounds or prepared mixtures.

- Methods:
 - The toxicity of the bulk sediment, collected at selected sampling stations and times, will be evaluated in the 10 day mortality with *Corophium* proposed by OSPAR (1995) and other international organizations.
 - The YES will be performed according protocols established in the ENDIS-RISKS project.
 - Similarly the experiments and assays to be conducted with *Neomysis* were developed within the context of this project and are extensively described by Ghekiere (2006a); endpoints which will be used are vitellin induction (ELISA, larval development, growth and molting.
 - The algal growth inhibition test with *Phaeodactylum* will be performed according to OECD guideline 201 (1984).
 - The fertilization and larval development assay with mussel or oyster will be performed according US EPA standard procedures. Briefly, sperm and eggs release from isolated laboratory female and male brood stock is stimulated through temperature increase. After mixing, the fertilized eggs allowed to develop for 48h after which the fertilization and development of the larva is scored microscopically (appearance of D-shaped larvae).
 - Assessment of the Scope for Growth (SfG) of *Mytilus* will be performed according to Widdows et al, 1995). Briefly in this assay the physiological responses (clearance rate, respiration rate and food absorption efficiency) of approximately mussels are measured after exposure to the contaminant mixture. Mathematical integration of these responses allow to calculate the potential of the mussels to growth (SfG). It has

been demonstrated this assay allows coupling of this sensitive stress response (scope for growth—SFG) and contaminant levels in the tissues of mussels and have consequently been suggested as a powerful and cost-effective method of assessing environmental pollution (Widdows et al., 1995; Widdows & Donkin, 1992).

- Assessment of lysosomal stability in *Limanda* liver will be performed according to the methods described by Moore (1990) and Köhler (1991), as proposed by OSPAR.

□ *In situ* field assays:

In essence, the test described above with mussels (larvae and adults), mysids (vittelin only) and dab (or flounder) will also be performed in the field. This will be accomplished through the use of dedicated cages deployed at the sampling sites (mainly inner harbour and at least once using mussels deployed for ecological assessment, see section 2.2.3). To this end test organisms will be collected at uncontaminated sites and transplanted (in cages) to the sampling stations. It is envisaged that this will be performed at least twice during the course of the project. After exposure (cf. above for exposure time), the organisms will be transferred to the laboratory and the selected test endpoints will be measured using the same methods as those applied in the laboratory exposure experiments.

□ Determination of bioaccumulation

Contaminant tissue concentrations of the laboratory- and field exposed organisms, together with some selected resident species (to be decided) will be measured. This will allow to assess the bioaccumulation of the contaminants. This information is essential for the determination of possible effects on human health through consumption of marine products and the evaluation of the effects and concentration measured in the coastal tern populations.

□ Expected results

The approach of using the same species in both laboratory and field exposure experiment combined with the novel laboratory exposure system (mixture of contaminants reflecting realistic field concentrations) can be considered as unique. If successful, this approach will not only dramatically change monitoring and assessment procedures but may also give insight into the relative importance and impact of the various hazardous substances present in the marine environment.

2.2.3. Task 3: Ecological monitoring

Target species for ecological monitoring: ecological relevance and importance

The species selected for ecological assessment/monitoring and biological evaluations within the framework of the establishment of relationships between local contaminant occurrence, ecotoxicological effects, ecosystem health and potential human health effects are: mysids, mussels, oysters and the common tern. The selection of these organisms is based on their abundance, relevance and ecological importance for the Belgian coastal ecosystem.

The inshore hyperbenthic community is characterized by high densities and biomasses, and consists nearly exclusively of mysids: *Schistomysis spiritus*, *Schistomysis kervillei* and *Mesopodopsis slabberi* (Dewicke et al., 2003). Mysid shrimps are important members of the food web of the coastal zone as they feed on detritus, algae or zooplankton (Mauchline, 1980) and contribute substantially to the diet of fish (Beyst et al., 1999; Hostens and Mees, 1999) and shrimp. Mysids are sensitive to some chemical contaminants at environmentally relevant concentrations and have been used for ecotoxicological testing in the context of ENDIS-RISKS. The experience with population structures of Mysida gained during ENDIS-RISKS, showed that a study of the population parameters are a possible useful tool in the frame of ecotoxicological research.

Most of hard substrata along the Belgian coast are artificial. These include breakwaters, dikes, harbour walls, even buoys form a particular artificial habitat. They harbour a community typical for a moderately exposed rocky coast with a high species diversity and biomass and they are characterized by dense mussel and oyster clusters. The pacific oyster (*Crassostrea gigas*) and the blue mussel (*Mytilus edulis*) are both frequently used to study levels and trends of chemical contamination in coastal waters. As filter-feeders these bivalves are particularly at risk to environmental contaminants as they are stationary, epibenthic, and filter relatively high volume of water, including suspended particles. Furthermore these bivalves do not only take an important place in the food web of hard substrata but are also of a high commercial value.

Coastal birds have proven to be an excellent monitoring system for evaluating chemical pollution. common terns (*Sterna hirundo*) can play a prominent role as bioindicator for the Belgian coastal zone. Since the 1980s the harbour of Zeebrugge hosts a very important breeding colony of the common tern. For example in 2005, almost 5 % of the biogeographical population was breeding in Zeebrugge, making it the largest colony in Europe with 3052 breeding pairs. As a top-predator the common tern feeds on fish occupying higher trophic levels making the former particularly vulnerable to accumulate persistent chemicals. Common terns feeding on contaminated fish may during the breeding season, transfer part of their contaminant burdens to their eggs, finally ending up in the hatchlings and thus potentially affecting the reproductive success of the population.

As such contaminant concentrations in tern eggs are a particularly important evaluation/monitoring endpoint as they both indicate the local pollution sources and at the same time provide information on long-term temporal trends in the contamination status of the reproductive females, thus revealing the health status of the coastal environment. Finally is worth mentioning that common terns attracts widespread public interest and is a vulnerable species protected through “Annex I of the Bird Directive” and “The Convention of Bern and Bonn”.

Ecological assessment methodology

□ Mysids and benthic fauna

Elaborating on the experience with population structures of Mysids gained during ENDIS-RISKS, mysid shrimp populations will be sampled, where relevant, at each of the sampling stations. From the sampled biota, density, diversity, population composition (sex and developmental stage) and fecundity will be determined. Elucidation of the population structure along the Belgian coast of mysid shrimps has previously never been executed, neither were they ever sampled in harbours.

Mysid shrimp populations will be sampled by means of a hyperbenthic sledge. Sampling will be done quantitatively in the Scheldt and in the coastal zone. In the harbours the mysids will be sampled qualitative. Other epi- and benthic fauna will be collected and analysed at least twice during the course of the project organisms using Van Veen grab samplers.

□ Blue mussel & Pacific oyster

In order to control the source, age and stage of sexual maturity of the mussels, cage experiments will be used. Cages will be placed along a gradient from the harbour through the coastal zone to the most offshore sampling stations located on the Belgian Continental Shelf. Various biometric parameters representing the somatic growth of the mussel can be recorded, such as: number of annual growth rings, length, width, height of the shell, and others. Dry weight of the soft tissue and of the shell will be measured to determine the condition index: a good indicator for shell growth. Age related changes in the indices can be approximated by the von Bertalanffy equation. Another biometric parameter which will be used in this research is the gametogenesis and the gonadal development, this can be examined by histological sections of the gonads (Seed, 1975). The SfG of mussels collected from these cage experiments will also be analysed (see 2.2.2.).

□ Sternidae

To analyze contaminant levels, a number of fresh eggs of the common tern will be sampled every year for a period of three years. The eggs of selected breeding pairs will be analyzed for pollutants. Additionally the reproductive success and development of chicks origination from those sample nests will be studied. This will be done by the INBO as they have a profound experience in studying breeding colonies of terns (Stienen et al., 2004; Stienen et al., 2002; Stienen et al., in press). The approach is based on the method “sample egg technique”, which provides hints to critical residues of pollutants that negatively affect nest success (Blus et al. 1974, Blus 1984, Henny et al. 1984, Becker et al. 1993).

□ Expected results

From the research with the mysids and the bivalves we expect to be able to detect differences in populations and/or biometric parameters depending on the differences of contaminations at the different study sites. These results will be coupled with the chemical monitoring and lab and field ecotoxicological data to link the results of the various tools deployed in this project with important ecological indicators of ecosystem health.

Similarly, for the Common tern we to be able to like measured biometric and population level parameters to the contaminant concentration in the eggs. This will allow assessing trophic transfer of the contaminated present in Belgian coastal and their impact on top-predators.

2.2.4. Task 4: Integrated risk assessment

The results of the components (tasks) will be incorporated into an integrated risk assessment for Belgian coastal waters (including harbours) of the selected contaminants. Using the results of the extensive chemical monitoring of ‘conventional’ and ‘new emerging’ micropollutant (task 1) combined with the novel laboratory and field effect assessments (task 2) and the ecological assessment endpoints (task 4) the impact of the presence of these contaminants on Belgian coastal on the health of marine ecosystems, important coastal bird populations and man (through consumption of marine products) will be assessed in a quantitative manner. Because of the novel approach used in this project it is anticipated that for the first time, the potential risks of mixtures of low level contamination by persistent micropollutants to marine ecosystems and man will be demonstrated and/or elucidated.

Additionally, it is expected that this research will provide information to assess the relevance and validity of the currently used procedures to derive Predicted No Effect Concentrations (EU, Existing Substance Directive and REACH) and/or Ecological Quality Criteria (OSPAR) and/or Environmental Quality Criteria established in the context of the EU WFD. The most recent (probabilistic) risk assessment techniques will be applied in conjunction with currently

used internationally accepted risk and impact assessment procedures (e.g. as described in the EU Technical Guidance Document, 2003).

2.2.5. Task 5: Development of assessment and management tools for the routine evaluation of the impact of micropollutants on the Belgian coastal zone and man: towards sustainable development

Based on the results and the in-depth evaluation of all components of the research project, we will develop (1) a toolbox of relevant and (ecologically) validated measurements and assay to be performed in the assessment of the environmental quality of coastal waters in general and that of the Belgian coast in particular; (2) a suite of transparent assessment procedures and evaluation criteria to allow to monitor (geographically and temporally) and make science-based environmental management decision.

Literature

- Becker P. H. et al. (1993). *Oecologia* 93: 389-393.
- Beyst B. et al. (1999). *J. Fish. Biol.* 55: 1171-1186.
- Blus L et al. (1974). *Environ. Pollut.* 7: 81-91.
- Blus L.J. (1984). *Wilson Bull.* 96: 268-276.
- Booij K. et al. (2002). *Chemosphere* 46(8): 1157-61.
- Bound P. and Vouvolis N. (2005). *Environ. Health Perspect.* 113 (12): 1705-1711.
- Dewicke A. et al. (2003). *J. of Sea Res.* 49: 27-45.
- Folsik N. et al. (2002). *J. Environ. Monit.* 4: 260-283.
- Ghekiere A. et al. (2006a). *Ecotox. Environ. Safe.* (accepted).
- Henny C.J. et al. (1984). *J. Wildl. Manage.* 48: 1-13
- Hostens K. and Mees J. (1999). *J. Fish. Biol.* 55: 704-719.
- Huckins J.N. et al. (2002). *Environ. Sci. Technol.* 36(1): 85-91.
- ICES, 2005. CM 2005/E:01 100pp.
- Köhler A. (1991). *Comp. Biochem. Physiol.* 100C: 123-127.
- Mauchline J. (1980). The biology of mysids and euphausiids. In: Blaxter, J.H.S., Russell, F.S., Yonge, M. (Eds.). *Advances in Marine Biology*. Vol. 18. Academic Press, London. 681pp.
- OSPAR (1995). *Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry*.
- OECD (1984). (Organization for Economic Cooperation and Development) *Algal growth inhibition test guideline No 201. OECD Guidelines for Testing of Chemicals*, Paris.
- Roose P. and Brinkman U.A.Th. (2005). *Trends Anal. Chem.* 24: 897-926.
- Seed R. (1975). *Pubbl. Staz. Zool. Napoli.* 39: 317-334.
- Smedes F. and Koning K. (2005). Passive sampling of hydrophobic contaminants with silicone rubber in concert with deployed mussels. Evaluation of 3 years monitoring in Dutch Marine waters.

Presentation on the Passive Sampling Training Workshop, 27 June – 6 July 2005 RIKZ-Haren, The Netherlands

Stienen E.W.M. and Brenninkmeijer A. (2002). *Ibis* 144: 567-576.

Stienen E.W.M. and Brenninkmeijer A. (in press). *J. Ornith.*

Stienen E.W.M. et al. (2004). Interacties tussen antropogene activiteiten en de avifauna in de Belgische zeegebieden. Rapport IN.A.2004.136. Instituut voor Natuurbehoud, Brussel. 54 pp.

Van der Oost R. et al. (2003). *Environ. Tox. Pharm.* 13: 57-149.

Verweij F. et al. (2004). *Chemosphere* 54: 1675-89.

Widdows J. and Donkin P. (1992). Mussels and environmental contaminants: bioaccumulation and physiological aspects. In: Gosling EM (ed) *The Mussel Mytilus*. Elsevier, Amsterdam, The Netherlands, pp 383-424.

Widdows J. et al. (1995). *Mar. Ecol. Progress Series* 127: 131-148.