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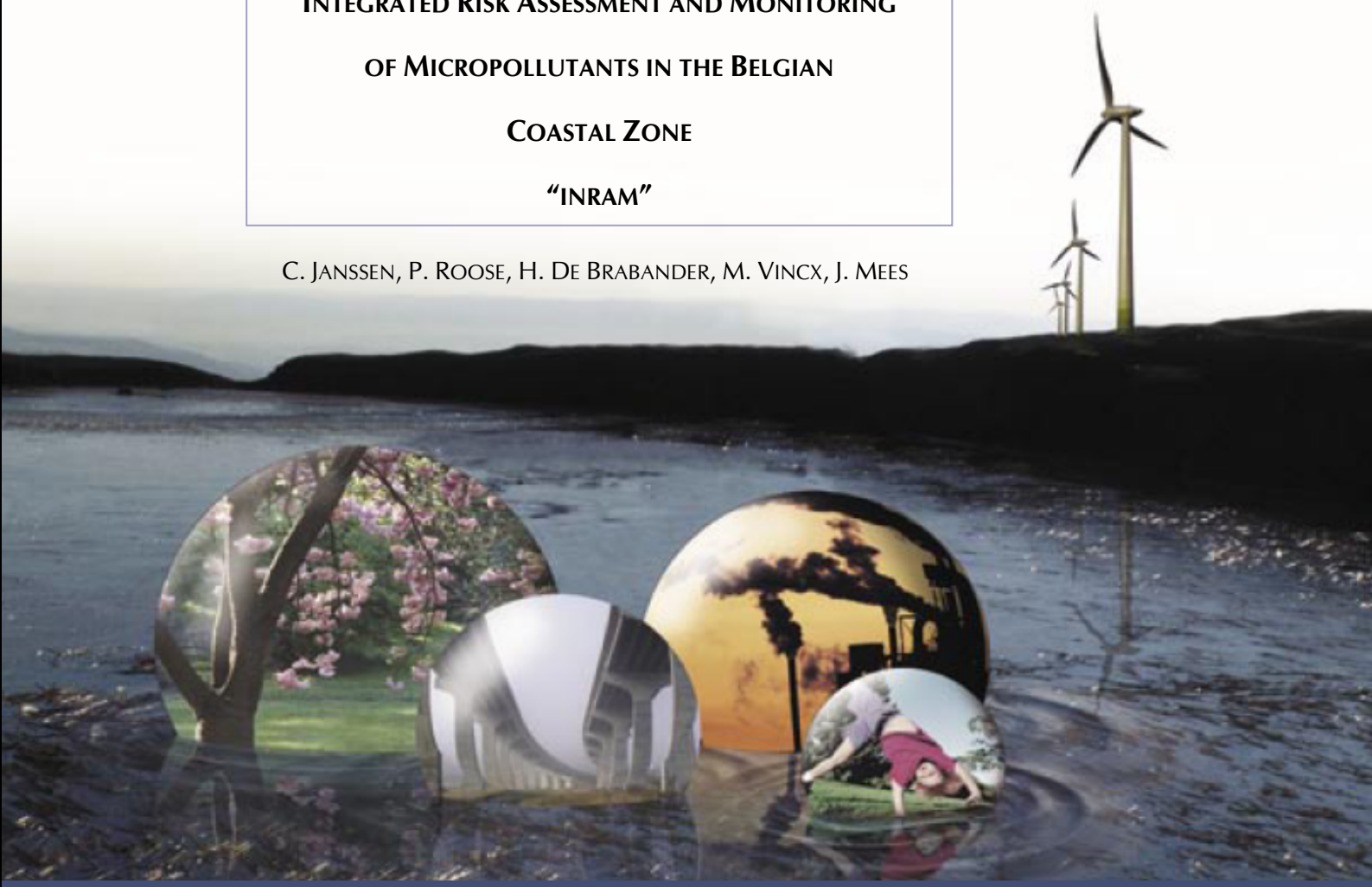
INTEGRATED RISK ASSESSMENT AND MONITORING OF MICROPOLLUTANTS IN THE BELGIAN COASTAL ZONE "INRAM"

C. JANSSEN, P. ROOSE, H. DE BRABANDER, M. VINCX, J. MEES



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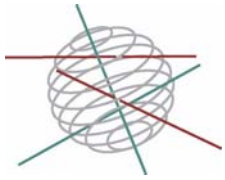

North Sea

FINAL REPORT PHASE 1

INTEGRATED RISK ASSESSMENT AND MONITORING OF MICROPOLLUTANTS IN THE BELGIAN COASTAL ZONE

"INRAM"

SD/NS/02A

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2 Abstract

In both the scientific and regulatory arena, there is currently general agreement that the best way to assess the health of the marine environment with respect to the adverse effects of hazardous substances, is through the use of a suite of chemical and biological measurements in an integrated fashion. An integrated monitoring approach will allow interpretation of biological effects data, taking account of processes such as bioavailability, effects at different levels of biological organisation, biomarker relevance, bioaccumulation, biomagnification and time-lags between the exposure and the emergence of effects. This will assist the final integrated assessment, which will investigate the links between observed effects and the concentrations of the hazardous substances.

In this regard, the INRAM project aims to develop an integrated risk assessment tool to assess the impact of micropollutants on the Belgian marine environment. Additionally, potential human health effects will be assessed through the use of consumer organisms as test species.

The first phase of the project can be divided into four different research tasks. In task I, the occurrence and distribution of micropollutants in the Belgian coastal zone was studied. During 2007, water, sediment, suspended solids and biota were sampled three times at 19 selected sampling stations in the three Belgian coastal harbours (11 stations), the Belgian continental shelf (6 stations) and the Scheldt estuary (2 stations). In 2008, the same stations were sampled once during an integrated summer campaign. In these matrices, several groups of chemicals were already analyzed: organonitrogen pesticides, pharmaceuticals, polyaromatic hydrocarbons (PAHs), polyaromatic components (PCBs, PBDEs), brominated flame retardants (BFRs), organotins, phenols and phthalates. All the analyzed chemicals are priority compounds (cfr. OSPAR, WFD and UNECE lists) and emerging pollutants (e.g. pharmaceuticals).
In Task II, the effects of the above contaminants on selected test organisms (relevant for the Belgian marine environment), were assessed either through single toxicant testing or mixture testing. For the latter, a novel technique using contaminated passive samplers was used, allowing test species to be exposed to complex pollutant mixtures at environmentally relevant and stable concentrations.
Task III deals with the assessment and monitoring of the ecological health of the Belgian marine environment through the use of dedicated *in situ* cage experiments with bivalves, combining ecotoxicological and ecological endpoints; and through population studies on hyper- and epibenthos samples collected during the integrated sampling campaigns.
Finally, in Task IV, a preliminary risk assessment was conducted based on the first results of the chemical analysis of Task I.

During the integrated sampling campaigns, water samples were collected with Teflon-coated Go-Flo bottles (10L); if the water depth was insufficient, Niskin bottles were used. Sediment samples were taken with a Van Veen grab, biota were sampled with a hyperbenthic sledge (for hyperbenthos), a beam trawl (for epibenthos) or a Van Veen grab (macrobenthos). Suspended particulate matter was sampled with an Alfa Laval flow-through centrifuge. For the chemical analysis of farmaceutical compounds in seawater, a new analytical method was developed. For the analysis of the other compounds in all the matrices, protocols from the preceding project ENDIS-RISKS were used.

Ecotoxicological experiments were conducted to assess the impact of the micropollutants present in the Belgian marine environment on the health of selected resident marine species. In this first phase of the project, the marine diatom *Phaeodactylum tricornutum* (72h growth inhibition test), the blue mussel *Mytilus edulis* (Cellular energy allocation (CEA) assay, ethoxyresorufin O-deethylase (EROD) assay) and the Pacific oyster *Crassostrea gigas* (24h larval development test) were used. Single toxicant tests were performed as well as mixture toxicity tests. For the latter, a new exposure technique using contaminated passive samplers was developed. This technique allows the exposure of test organisms to complex mixtures of micropollutants at environmentally relevant and stable concentrations under controlled laboratory conditions. Finally, a limited and preliminary Quantitative Toxicological Interpretation (QTI) analysis was performed to unravel the toxic composition of the mixtures.

For the ecological health assessment and monitoring, biota samples (hyperbenthos, epibenthos and macrobenthos) were collected as described above. On these samples, population structure studies were performed. Field experiments with caged bivalves (mainly *Mytilus edulis*, collected in the Eastern Scheldt from subtidal mussel plots) were conducted, in which the organisms were deployed for six weeks in the Belgian coastal harbours and at one station in open sea. Three ecological endpoints (Shell Length, Shell Length Increment and Condition Index) were monitored through time. Simultaneously with the last cage experiment (October – November 2008), passive samplers were deployed to collect the local micropollutants for subsequent chemical analysis and for the ecotoxicological assays as described above. These will both be conducted in the second phase of the project, as will the chemical analysis of the tissue of the exposed organisms. For this last cage experiment, the analysis of a battery of five ecotoxicological biomarkers (Cellular Energy Allocation, Lysosomal Stability, EROD activity, Metallothionein content and Alkali Labile Phosphates) is also planned. The EROD activity and CEA have already been determined.
A preliminary risk assessment was performed according to the REACH Implementation Project (RIP). Data on physico-chemical parameters, toxicological dose descriptors and ecotoxicological endpoints were gathered through different sources. Subsequently, Risk Characterization Ratios (RCRs) were derived for selected chemicals by comparing exposure levels (based on the chemical analysis from Task I) to suitable Predicted No Effect Concentrations (PNECs) or derived no-effect levels (DNELs) for environmental and human health endpoints respectively.

The results of the chemical analysis show that multiple micropollutants are present and that multiple Environmental Quality Standards (EQS) and Environmental Assessment Criteria (EAC) were exceeded. Concentrations of PAHs in sediments regularly exceeded the lower limit of the Ecological Assessment Criteria (EAC) for PAHs (stations OO1, OO2 and S22) although the upper limit of the EAC was never exceeded. The EAC was most frequently exceeded for pyrene. The compounds anthracene, fluoranthene and benzo(a)pyrene exceeded the EAC for PAHs in water in respectively 49%, 10% and 13% of the cases with a factor ranging from 1 to 16. The Annual Average Environmental Quality Standard (AA-EQS) (WFD directive 2000/60/EC), for water was exceeded for sum(BkF, Indeno) and sum(BbF, Bghi) in respectively 85% (factor 1-15) and 10% (factor 1-3) of the cases. At S22 and OO2 the EQS was exceeded with a factor of 30. As the EAC (Ospar, 2000) for TBT in sediments is set on 0.05 $\mu g.kg^{-1}$ dry weight, it was exceeded in all stations with a factor 1 to 1600. The AA-EQS (0.2 $ng.L^{-1}$) and EAC (0.1 $ng.L^{-1}$) for TBT in water were exceeded in 79% of the cases. Also, the maximum allowable EQS (1.5 $ng.L^{-1}$) was exceeded in

47% of the cases. AA-EQS for penta-BDEs is 0.2 $ng.L^{-1}$ and was exceeded in 83% of the cases in this study. For nonylphenol, the EQS was exceeded at three sampling locations in May 2007. For the organonitrogen pesticide (ONP) diuron the EQS was exceeded at sampling locations NP1 (Nieuwpoort harbour), S22 (Scheldt estuary in Antwerp) and OO2 (Oostende harbour); for terbutylazine, the considered PNEC value was exceeded at station S22 and OO2 in April 2008. Pharmaceuticals were detected up to 0.855 $\mu g.L^{-1}$. To the best of our knowledge, this is the first time pharmaceuticals were found in the marine environment at these concentration levels. These results confirm the presence of pharmaceuticals in the North Sea and their ubiquitous character.

Contaminated passive samplers were successfully used as a source of complex micropollutant mixtures originating from the Belgian coastal harbours and the North Sea, in laboratory ecotoxicological experiments. Effects of these mixtures were observed on the larval development of oyster larvae (*Crassostrea gigas*, 24h larval development test) and on the growth of the marine diatom *Phaeodactylum tricornutum* (72h growth inhibition test). *C. gigas* was by far the more sensitive organism, showing adverse effects in all harbours. Clear differences could be observed between the harbours: in Oostende (OO2) and Zeebrugge (ZB2), up to 100% and 83% mortality was observed respectively; in Nieuwpoort, no more than 17% mortality was found, up to 70% abnormal larvae were observed. *P. tricornutum* exhibited growth inhibition only at station OO2. Results of the preliminary QTI, imply that the observed effects on *P. tricornutum* are mainly cause by phenol pollution. The main cause of abnormality (and mortality) of oyster larvae, are organotins and PAHs.

A first cage experiment with the mussels and oysters revealed clear differences in overall fitness of marine bivalves within a gradient from outer harbour to inner harbour. A second experiment showed a clear difference between the bivalves exposed at a sea station in comparison with the bivalves exposed at the marinas of the different harbours. Moreover, the results of the cage experiments show more severe effects on the overall fitness in the more polluted areas (e.g. the Sluice Dock of Oostende and the Yacht Harbours/Marinas). Preliminary results of the analyses of the macrobenthos diversity show a difference in species diversity of the brackish stations in the inner harbours in comparison with the outer harbour stations which, on their turn, are slightly different from the sea stations.

The preliminary risk characterization didn't reveal any risk at the respective sampling locations for nonylphenol, atrazine, diuron and isoproturon for the sampling periods of May and June 2007 and December 2007.

A good agreement between local pollution observed through the sampling campaigns and subsequent chemical analysis, and the local health status of the marine ecosystems observed through ecological and ecotoxicological laboratory and field experiments is already apparent from the results of the first phase. Perhaps one of the most crucial observations is that, even though preliminary, the performed risk characterisation did not reveal any chemicals that are posing a risk according to the current evaluation methods. Yet, the ecological and ecotoxicological studies conducted in this first phase of the project, do show clear pollution-related adverse effects. This confirms that complex mixtures of pollutants can cause adverse effects on organisms, even when the individual components of the mixture are present at concentrations well below their effect levels.

3 Introduction

The marine environment receives inputs of hazardous substances through riverine sources (including harbours), direct discharges and atmospheric deposition. As a result, a large number of anthropogenic chemicals is present in the marine environment. This means that the organisms living in these ecosystems are exposed to a range of substances which have the potential to cause metabolic disorders, increases in disease prevalence and population effects such as changes in growth, reproduction and survival.

The presence of persistent anthropogenic chemicals in our marine environments is not a new problem. Since the 1960s, an increasing number of environmental contaminants have been detected and their reported concentrations have been a source of continuous scientific, governmental and public concern. Numerous types of compounds have been identified and have been the subject of intensive (mainly chemical) research efforts. During the last decade for example, compounds which affect the hormone systems of animals and their offspring, the so called endocrine disruptors, have attracted the world-wide attention of researchers and regulatory bodies such as the European Union (EU) and the Oslo and Paris Commission (OSPAR). Some of these chemicals, such as PCBs, dioxins and organotins, have been well studied, while for others such as flame retardants and many surfactants much less is known (Darnerud et al., 2001, Palm et al., 2002 and Ying et al., 2002). All of these chemicals have, however, been frequently detected in high levels in the sediments and waters of estuarine environments world-wide (Fent, 1996, de Voogt et al., 2000, Darnerud et al., 2001 and Ying et al., 2002). Unfortunately, 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 (DeFur *et al.*, 1999).

Despite extensive research efforts, 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. Some of the main reasons for this are: **(1)** monitoring of these chemicals is usually based on chemical measurements in water and to a lesser extent in other matrices (e.g. sediments or biota); **(2)** the assessment of the potential effects of these compounds is mostly performed for a single chemical at a time and with a limited number of standard (and often environmentally irrelevant) species under laboratory conditions; **(3)** both in these laboratory tests, and in the very rare field-experiments, the biomarkers used as evaluation endpoints mostly have an unknown relevance for predicting/evaluating ecosystem level effects; and **(4)** measurements using field-collected (exposed) species using the same type of biomarkers suffer from the same shortcoming.

In both the scientific and regulatory arena, there is currently general agreement that the best way to assess the health of the marine environment with respect to the adverse effects of hazardous substances, is through the use of a suite of chemical and biological measurements in an integrated fashion. An integrated monitoring approach will allow interpretation of biological effects data, taking account of processes such as bioavailability, effects at different levels of biological organisation, biomarker relevance, bioaccumulation, biomagnification and time-lags between the exposure and the emergence of effects. This will assist the final integrated assessment, which will investigate the links between observed effects and the concentrations of the hazardous substances.

The INRAM project builds upon the developed know-how of the ENDIS-RISKS project (Janssen *et al*. 2008) to establish an enlarged (compounds analyzed and tested not limited to endocrine disruptors, 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 INRAM project are to (1) study the environmental concentrations of established priority compounds (cf. OSPAR, WFD and the UNECE lists) and emerging pollutants (e.g. pharmaceuticals), and their transfer via the three Belgian coastal harbours and the Scheldt, to coastal waters; (2) apply a unique combination of novel field and laboratory ecotoxicological and chemical techniques to establish both effects and food chain transfer of these chemicals; (3) for the first time, 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.

The first phase of the INRAM project can be divided into four main parts:

3.1 Exposure assessment – sampling and chemical analysis

A total of 17 sampling stations in the three Belgian coastal harbours and on the Belgian Continental Shelf (BCS), and 2 additional sampling stations located in the Scheldt river (see Figures 1&2), were sampled three times in 2007, once in 2008. On each sampling station, water, sediment, biota and suspended particulate matter (SPM) were sampled.

For each harbour, one sampling station was situated in the inner harbour near industrial or boating activities (e.g. pleasure crafts) and one station near the harbour mouth. At least one additional station was strategically chosen to allow the assessment of potential pollutant gradients within each harbour. One monitoring station was 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 were selected: i.e. one near the city of Antwerp (Endis-Risks station No. 7) and one near the mouth of the estuary (Endis-Risks station No. 1, Vlissingen).

Next to these 13 harbour/river stations, an additional 6 sampling locations along the Belgian coast were selected. For each harbour, one was located near the harbour mouth, a second station further off-shore (at least 5 kilometres from the coast) to assess the contaminant gradient.

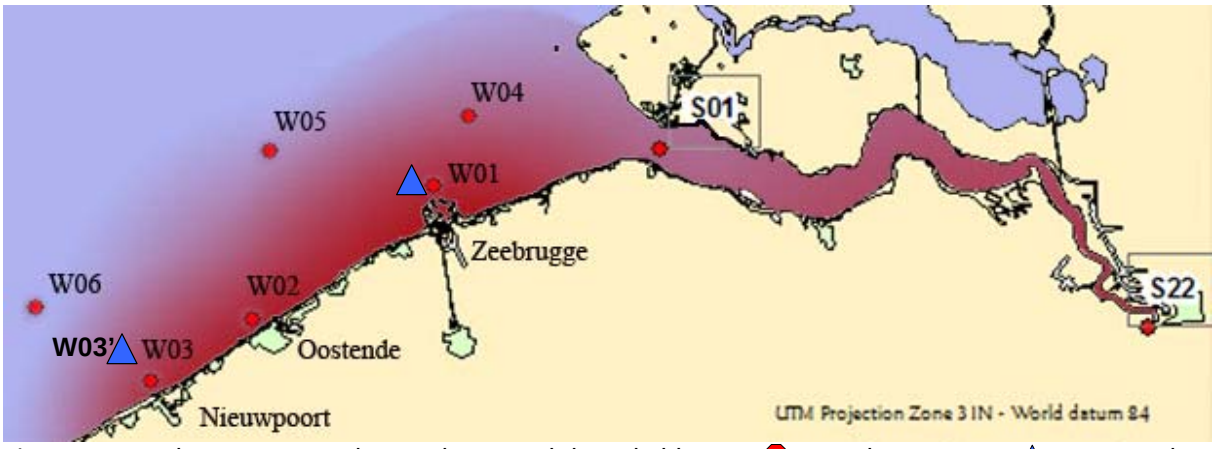

Figure 1. Sampling stations in the North Sea and the Scheldt river. ●: sampling station. ▲ : cage with passive samplers and/or bivalves deployed.

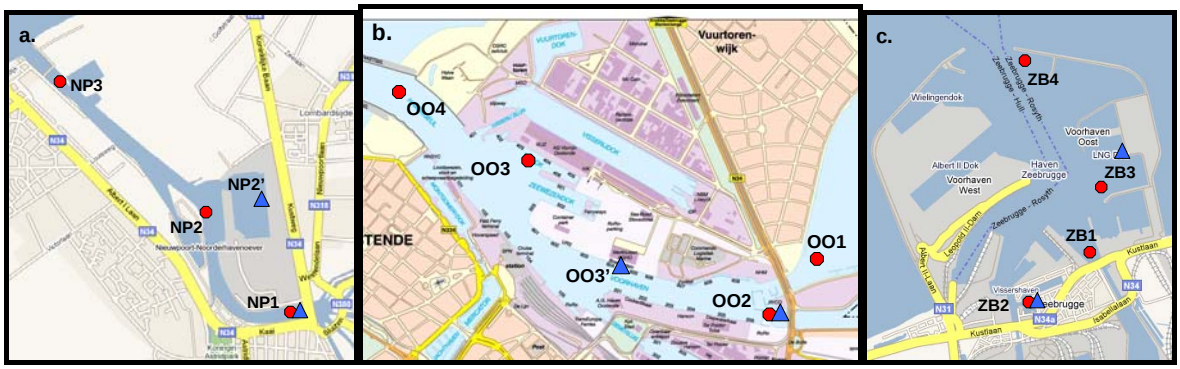

Figure 2. Sampling stations in the harbour of (a) Nieuwpoort, (b) Oostende and (c) Zeebrugge. ● : sampling station. ▲ : cage with passive samplers and/or bivalves deployed.

All samples collected during the sampling campaigns, were chemically analyzed using chemical analytical procedures previously established in the partners' laboratories (cf. ENDIS-RISKS network). Next to the above mentioned 'conventional' sampling and analytical techniques, the use of passive samplers in the context of marine monitoring and ecosystem health assessment is being further developed and validated. This novel approach is central to several of the innovations envisaged to result from this project. Passive samplers made of polydimethylsiloxane (further referred to as PDMS sheets) will be used in the context of (a) chemical analysis, to aid the analysis of hydrophobic compounds present at concentrations below conventional detection limits in the marine environment; (b) as a surrogate for biota to assess bioavailability and bioaccumulation, eliminating the need for time consuming and expensive chemical analysis of organic tissue; and (c) as a contaminant source for dedicated ecotoxicological (laboratory) assays allowing exposure of organisms to environmentally relevant chemical mixtures.

The selection of chemical compounds that was analyzed is based on the experience on the analyses and occurrence of these compounds gained by the research consortium 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 were still included, partly because they are on the UNEP-POP list but also

because of their environmental relevance. Tributyltin and some selected pharmaceuticals were also analyzed. The compounds of this latter group are: four antibiotics (sulfamethoxazole, ofloxacin, trimethoprim, chloramphenicol), five non-steroidal anti-inflammatory drugs (mefenamic acid, diclofenac, salicylic acid, paracetamol, ketoprofen), two β-blockers (propranolol, atenolol), two lipid regulators (bezafibrate, clofibric acid), one psychiatric drug (carbamazepine) and one β-agonist (salbutamol). 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 Vouvoulis, 2005). This selection of pharmaceutical compounds is based on a in-depth literature analysis and information on their use in Belgium. Analytical methods for the quantification of these compounds in seawater have been developed based on the available literature and the experience of one of the project partners with the analysis of veterinary products.

3.2 Effect and bioaccumulation assessment

To assess the effects of the contaminants present in the Belgian marine environment, a set of organisms was exposed to either individiual toxicants ('priority compounds' selected based on the results of the first sampling campaigns) or environmental pollutant mixtures in dedicated ecotoxicological experiments. Effects of (mixtures of) contaminants on (1) the growth rate of a marine diatom (*Phaeodactylum tricornutum*), (2) the development of pacific oyster larvae (*Crassostrea gigas*) and (3) biomarker responses of adult blue mussel *Mytilus edulis*, were studied. A novel exposure technique using PDMS sheets as a passive dosing device, allowed exposure of the organisms to mixtures closely resembling true environmental pollutant mixtures. These contaminated devices were used as an exposure source in laboratory assays with the above species. The concentrations of the components of the contaminant mixture released from the samplers into the bio-assay vessels reflect *in situ* exposure concentrations and are constant in time. As such relevant lab to field comparisons and extrapolations are feasible.
Field assays using caged mussels (*Mytilus edulis*) were used to assess the effects of contaminants in field situations. A battery of five biomarkers was used to allow interpretation of the observed effects based on the occurrence of contaminants in the local environment. Simultaneously, PDMS sheets were deployed in order to absorb the contaminants present in the local environment, allowing the ecotoxicity assessment of the local environmental pollutant mixture in the growth inhibition test with *P. tricornutum* and the larval development test with *C. gigas* larvae.

3.3 Ecological monitoring

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 effects are: mussels, oysters, mysid shrimps and the common tern. The selection of these organisms is based on their abundance, relevance and ecological importance for the Belgian coastal ecosystem.

Cage experiments with transplanted blue mussels (*Mytilus edulis*) and pacific oysters (*Crassostrea gigas*) were conducted to study biometric parameters (growth, condition indices and gonadal development) of the organisms in relation to contaminants present in the field. A first experiment

was set up to study a gradient from an inner harbour to an outer harbour. A second more elaborate experiment elucidated the difference between harbour stations and an open sea station.

Ten eggs of the common tern (*Sterna hirundo*) were collected every spring from a breeding colony in the harbour of Zeebrugge. The eggs are being analyzed for pollutants. Information of the reproductive success and the development of the chicks originating from that colony are available.

Beside the study of the focus organisms, epibenthic, macrobenthic and hyperbenthic fauna was sampled, if practically possible (see 5.3), on all 19 stations. As such, a gradient in density and diversity for the different ecological compartments of the benthos could be assessed, in relation with a possible contaminant gradient, from the inner harbours to the open sea.

3.4 Preliminary risk assessment

In the first phase of the project, an extensive literature search has been conducted to gather all information needed to perform a preliminary risk assessment. Data on physico-chemical parameters, toxicological dose descriptors and ecotoxicological endpoints of the selected micropollutants were gathered through different sources. A preliminary risk characterisation was performed with the available results of chemical analysis, i.e. nonylphenol and some organonitrogen pesticides (atrazine, diuron and isoproturon) for the aquatic compartment (inland and/or marine).

4 Materials and methods

4.1 Exposure assessment

4.1.1 Sampling (water, sediment, suspended solids, biota)

Samples in the three coastal harbours were collected onboard the rigid inflatable vessel (RIV) '*Zeekat*'. For the coastal stations and the Sheldt stations (see Figures 1&2), either the RV '*Belgica*' or the RV '*Zeeleeuw*' was used.
Upon arrival at the sampling location, an STD (salinity – temperature – depth) profile was made, immediately followed by water sampling. Samples for chemical analysis were collected with Teflon-coated Go-Flo bottles (10L) at a depth of 3m. At stations where the depth was insufficient for Go-Flo bottles (a depth of at least 10m is required), a Niskin bottle was used. The latter was also used to take samples for the measurement of supporting inorganic parameters.
Next to the collection of samples for chemical or biological analyses, temperature, salinity, conductivity and dissolved oxygen were also measured during sampling. Additionally, oceanographic, meteorological, navigational and other relevant data were continually collected using measurement devices onboard the respective research vessels.

4.1.2 Chemical analysis

4.1.2.1 Conventional methods

4.1.2.1.1 Organotins, PAHs, PCBs, OCPs

Each chemical compound group required a specific approach for chemical analysis. All methods for analysis were optimised and validated to reach the detection limits required to analyse the low environmental concentrations studied in this project. Sample intake, sample concentration and sample injection were three important parameters that were adjusted to enhance the sensitivity of the analysis. What follows is a short description of the used methods. More specific information is available within the consortium.

Sample preparation

Sediment samples were centrifuged to obtain the clay fraction ($< 63\ \mu$m) using a flow-through centrifuge (Biofuge Stratos Heareus, Kendro Laboratory Products, Hanau, Germany). Biota samples were mixed by a dispersion tool (IKA-Ultra-Turrax® T25 basic, Staufen, Germany). Solid material samples were then freeze dried with a Christ LMC-2 (Osterode, Germany), milled and homogenized with a Fritsch Pulverisette (Idar-Oberstein, Germany). The samples were stored at -20°C prior to further analysis.

Sample extraction

For analysis of PAHs, PCBs, PBDEs and OCPs a single extraction method is used. 1 to 5 L water samples were extracted using solid-phase extraction with Bakerbond Speedisk C_{18} extraction cartridges (JT Baker, Deventer, The Netherlands). 1 to 20 g of sediment or SPM was extracted

with pressurised liquid extraction (ASE 200, Dionex, Sunnyvale, CA, USA) for analysis of PAHs, PCBs, PBDEs, OCPs. For analysis of organotins liquid-liquid extraction was used. Derivatisation of organotin compounds was done using sodium tetraethylborate (Sigma-Aldrich, Steinheim, Germany).

Sample clean-up

Co-extracted material was removed by adsorption chromatography on alumina with 5% or 10% (depending on the target analyte) deactivated AlOx (JT Baker, Phillipsburg, USA). Compounds were eluted with hexane and the extract was concentrated to 1 ml prior to GC-analysis using an evaporative solvent reduction apparatus (Zymark TurboVap II; Zymark, Hopkinton, MA, USA).

GC-analysis

Analysis of the PAHs, PCBs, PBDEs, OCPs and organotins is done separately for each compound group. A PTV-large volume injection (Programmed Temperature Vaporising) method was optimised per compound group, with injection volumes up to 70μL. For that purpose a glass sintered liner was used combined with a BEST PTV Injector (Thermo Electron Corporation, Austin, TX, USA). The analytical system consisted of a Trace GC fitted with a Combipal autosampler (CTC Analytics, Switserland). Separation of compounds was done on a fused silica capillary column coated with a siloxane stationary phase, the choice of the stationary phase depending on the compound group. Detection of PAHs and organotins was done with a mass spectrometric (MS)-quadrupole detector operated in the selected ion monitoring (SIM) election-ionisation mode (EI), while detection of PCBs, PBDEs and OCPs was done with an ion-trap MS (ThermoFinnigan, Austin, USA) in EI-MS-MS mode.

4.1.2.1.2 Phenols

Detection of phenols in water was executed in cooperation with the FEA (Laboratory Organic Micropollutants, Gent). The FEA has developed a quantitative method for detection of these compounds in water samples. Sample preparation included derivatisation of the phenols with pentafluorobenzoylchloride and extraction with hexane. Detection of the phenols was performed by gas chromatography (GC) and negative chemical ionisation mass spectrometry (MS) (6890N GC coupled to a 5973 MS, Agilent technologies, USA). This method is applicable in a concentration range of $\pm$ 0,2 to 4,0 μg.L^{-1}.

4.1.2.1.3 Organonitrogen pesticides

Detection of the organonitrogen pesticides (ONPs) in water was carried out in cooperation with the Flemish Environmental Agency (FEA, Laboratory Organic Micropollutants, Gent). This agency has developed a quantitative method for detection of these compounds in water samples. In brief, sample preparation consisted only of filtration, to eliminate impurities like humic acids and to avoid obstructions. Analysis of the filtered samples was performed by on-line solid phase extraction (SPE) coupled to liquid chromatography tandem mass spectrometry (LC-MS). The filtered sample was injected onto the precolumn (RP-C18) (10 μm, 10 x 2.1 mm, MZ-Analysentechnik GmbH, Mainz, Germany), which retained the targeted molecules. The ONPs were eluted on-line onto the connected analytical column (Hypersil ODS, 3 μm, 125 x 2.1 mm,

MZ-Analysentechnik GmbH, Mainz, Germany) and were detected with a mass spectrometer (1100 series, LC/MSD, Agilent Technologies, USA).

4.1.2.1.4 Pharmaceuticals

The developed analytical approach includes extraction of water samples using solid phase extraction (SPE) and identification by liquid chromatography tandem mass spectrometry (LC-MS). The described extraction technique was adapted from Gómez et al. (2006) and Nebot et al. (2007). Prior to extraction, the pH of the water samples was adjusted to 7 ± 0.5 using solutions of HCl and NaOH (1 M). The internal standard isobutcar 61 was added to every sample obtaining a concentration of 0.05 μg.L^{-1}. Water samples of 1 L were filtered through Whatman filter paper (GF/C Ø 47 μm, Merck, Darmstadt, Germany) in order to avoid clogging of the sorbent. Subsequently, filters were washed with methanol to prevent for losses of the compounds of interest. Solid phase extraction was carried out using Chromabond HR-X cartridges (3 ml, 200 mg, Macherey-Nagel GmbH & Co. KG, Düren, Germany). The cartridges were preconditioned with 5 mL methanol and 5 mL water. Samples were subsequently loaded and afterwards the cartridges were rinsed twice with 5 mL water. Next, the cartridges were dried under vacuum for 30 minutes. Elution was performed using 5 ml acetone and 2 x 5 mL methanol. The extracts were taken to dryness using a gentle stream of nitrogen and were reconstituted in 150 μL acetonitrile and 150 μL 0.02 M formic acid in water and finally transferred in LC-MS vials. 20 μL was injected into the LC-MS-system.

Analysis was carried out using a high performance liquid chromatography (HPLC) apparatus comprising an 1100 series quaternary gradient pump and autosampler (Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was achieved using a NUCLEODUR C18 Isis HPLC column (5μm particle size, 250 × 4.0 mm; Macherey-Nagel GmbH & Co. KG, Düren, Germany). A filter (porosity 2 μm, 4 mm; Alltech, Waukegan, IL, USA) and pre-column (Nucleodur C18, Isis, Macherey-Nagel GmbH & Co. KG, Düren, Germany) was used to prevent rapid deterioration of the analytical column. According to Gómez et al. (2006) the mobile phase consisted of a mixture of (A) 0.02 M formic acid in water and (B) acetonitrile. A linear gradient of 0.3 ml.min^{-1} was used starting with a mixture of 60% A and 40% B for 5 min. The acetonitrile percentage was increased from 40% to 100% B in 20 min (hold 10 min). Between each sample the column was allowed to equilibrate at initial conditions for 10 min. Analytes were detected with a LCQ DECA Ion Trap mass spectrometer equipped with an electrospray ionisation interface (ESI) (ThermoFinnigan, San José, CA, USA) in the negative MS-MS ion mode for chloramphenicol, salicylic acid, bezafibrate, clofibric acid and mefenamic acid and in the positive MS-MS ion mode for the other compounds considered. Multiple mass spectrometry was used to obtain structural information on the compounds corresponding to each chromatographic peak and to fulfill the criteria as described in European Criteria 2002/657.

4.1.2.1.5 Phthalates

Detection of phtalates in water was executed in cooperation with the FEA (Laboratory Organic Micropollutants, Gent). Phtalates could be determined quantitatively using liquid-liquid extraction (LLE), stir-bar-sorptive extraction (SBSE) and detection by GC-MS. Water samples were extracted by adding methanol and placing a stir bar (Twister, PDMS coated, Gerstel GmbH) in a

water sample (10 ml). By stirring for 60 min at room temperature the phtalates were absorbed on the stir bar through LLE and SBSE. Subsequently, the stir bars were introduced in a thermal desorption tube and desorbed (35°C to 300°C at 60°C.min^{-1}) in a thermal desorption unit (TDS 2, Gerstel GmbH). The desorbed compounds were cryofocused in a cold injection system (CIS, Gerstel) at -50°C. After desorption the compounds were injected into the analytical column of a GC-MS (6890N GC coupled to a 5973 MS, Agilent technologies, USA) by increasing the temperature of the CIS from -50°C to 300°C at 10°C.s^{-1}, which was then held for 5 min. Separation was performed on a 30m HP-5MS column with an ID of 0.25 mm i.d. and a film thickness of 0.25 μm (Agilent technologies, USA). The oven temperature was programmed to increase from 50 to 320 °C (hold 1.5 min) at a rate of 15°C.min^{-1}. Helium was used as carrier gas at a flow rate of 1 ml.min^{-1}. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron impact ionization at 70 eV.

4.1.2.1.6 Perfluorinated compounds

An analytical method that enables the detection of 13 perfluorinated compounds (PFCs) in water was developed in cooperation with the FEA (Laboratory Organic Micropollutants, Gent). The analytical approach included solid phase extraction (SPE) and detection using liquid chromatography (LC) coupled to a 'Time of Flight' – mass spectrometer (ToF/MS).

The analytical approach is based on a method developed by the Environment, Nature and Energy Department of the Flemish government. C^{13}-labeled internal standards were added to every sample (50 ml) obtaining a concentration of 0.100 μg.L^{-1}. Solid phase extraction was carried out using OASIS HLB (6cc, 200mg, Waters, Milford, MA, USA). The cartridges were preconditioned with 2 mL methanol and 2mL water. Samples were subsequently loaded and afterwards the cartridges were rinsed with 2 mL water. Elution was performed using 2 x 2 mL methanol. Next, extracts were dried to 0.5 mL using a gentle stream of nitrogen and 0.5 mL of 2.5 mM ammonium acetate in water was added. The extracts were finally transferred in LC-MS vials and 20 μl was injected into the LC-ToF/MS-system.

Analysis was carried out using a high performance liquid chromatography (HPLC) apparatus comprising an 1200 series quaternary gradient pump and autosampler (Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was achieved using a Luna C18 HPLC-column (5 μm, 2 x 250 mm, Phenomenex, USA). The mobile phase consisted of a mixture of (A) 2.5 mM ammonium acetate in water and (B) methanol. A linear gradient of 0.3 mL.min^{-1} was used starting with a mixture of 50% A and 50% B. The acetonitrile percentage was increased from 50% to 90% B in 10 min (hold 6 min). In 1 min the mobile phase returned to initial conditions (hold 6 min). Analytes were detected with a 'time of flight' mass spectrometer (LC/MSD TOF, Agilent Technologies, USA) equipped in the negative ion mode.

4.1.2.2 Novel approach – Passive sampling

Polydimethylsiloxane sheets (55mm x 90mm x 0.5mm; AlteSil Laboratory Sheet, Altec, Bude, England) were selected as passive samplers based on the properties of this material (Rusina *et al.*, 2007). Before use the sheets were pre-extracted for 100 hours with ethylacetate. Subsequently the sheets were spiked with performance reference compounds (PRCs) and stored

in glass jars with methanol until deployment. A set of 20 sheets was deployed on a stainless steel cage (with mussels) for a period of 6 weeks.

After deployment the sheets were dried and cleaned with a tissue and stored in the freezer until further use. For analysis sheets were extracted for 6 hours with a soxhlet extractor using 1:3 acetone-hexane solution. The extract was concentrated to 1 mL using an evaporative solvent reduction apparatus (Zymark TurboVap II; Zymark, Hopkinton, MA, USA). A clean-up over 4 g of alumina was done and the eluate was again concentrated to 1 ml and used for further analysis on GC-MS as described under 4.1.2.1 Concentrations could be expressed in water concentrations using diffusion and kinetic models (Huckins *et al.*, 2006).

4.2 Effects and bioaccumulation assessment

4.2.1 *Phaeodactylum tricornutum* 72h growth inhibition test

The *P. tricornutum* 72h growth inhibition test was performed according to ISO standard 10253 (ISO 2006). Briefly, test medium was prepared by spiking the right amount of toxicant stock solution (prepared either in methanol or ethanol) in sterilized artificial seawater (SASW). The SASW was prepared according to ISO standard 10253 (ISO 2006). Tests were performed in 100 mL Erlenmeyer flasks. Both controls and test flasks were inoculated with 10^4 cells mL^{-1} as initial cell concentration. All flasks were incubated for three days (72h) at 20°C and were shaken manually three times a day. Every 24h, algal growth rate was measured by counting the cells with a Beckman Coulter Counter.

4.2.2 *Crassostrea gigas* 24h larval development test

The *Crassostrea gigas* 24h larval development test was performed according to ASTM (1995). Briefly, test medium was prepared by spiking the right amount of toxicant stock solution (prepared either in methanol or ethanol) in artificial seawater (ASW). The ASW was prepared according to ASTM (1995). Both male and female oysters were induced to spawn by thermal stimulation. When spawn induction failed, gametes were stripped manually. Fertilisation was done by adding 10 mL of sperm suspension to 490 mL of egg suspension. The embryos were kept at 24°C at all times. Approximately one hour after fertilisation the embryos were spiked to the test solutions at an embryo concentration of 200 eggs/mL. For each concentration, three replicates were used. The test solutions were incubated at 24°C. After 24h, formaldehyde was added to the test solutions to terminate the test. The number of normally and abnormally developed larvae was determined under a light microscope. The EC50 was calculated using the trimmed Spearman-Karber method (Hamilton *et al.*, 1977).

4.2.3 *Mytilus edulis* biomarker analysis

In situ biomarker experiments with the blue mussel *Mytilus edulis* were conducted in the harbours of Nieuwpoort, Oostende and Zeebrugge. *Mytilus edulis* were collected from an uncontaminated site in the Oosterschelde and transplanted in cages to the three harbours. For biomarker analysis, organisms with a size of 4.5 – 5 cm were used. For ecological parameters (see 5.3.) and chemical analysis, organisms with a size of 3.5 – 4 and 4 – 4.5 cm were used

respectively. The cages were deployed in the harbours simultaneously for 6 weeks. Abiotic parameters were recorded three times during the course of the experiment. After recollection, the organisms were taken tot the laboratory on ice and dissected upon arrival. The digestive glands and gonads were frozen in liquid nitrogen and stored at -80°C. Organisms for chemical analysis were stored at -20°C.

4.2.3.1 EROD biomarker

For the analysis of EROD activity, the digestive glands of the organisms were cut in half. One half was used to determine total protein content using the Bradford method (Bradford, 1976). The other half was used to determine the EROD activity. For this, a protocol was developed based on the available literature.
Digestive glands were homogenized on ice in 200 μL of a homogenate buffer containing 100 mM Tris-HCl, 250 mM sucrose, 1mM EDTA and 1mM PMSF. After homogenisation, an additional 200 μL of homogenate buffer were added after which the samples were stirred. Subsequently the samples were centrifuged at 20.000g for 15-30 minutes. After centrifugation, 125 μL of a reaction buffer containing 50 mM Tris-HCl, 5mM $MgCl_2$, 10 mM NADPH, 10 μM of ethoxyresorufine and 10 μM of Dicumarol, was added to 50 μL of supernatans in a multiwell plate. Subsequently, the plate was incubated at 30°C. After one hour, the reaction was stopped by addition of 80μL NaOH. Resorufine concentrations were measured with fluorescence spectrometry at an excitation wavelength of 520 nm and an emission wavelength of 590 nm.

4.2.3.2 CEA biomarker

The goal of the cellular energy allocation assay (CEA) is to determine the available energy (Ea = protein + carbohydrate + lipid content) and the energy consumed (Ec, by means of electron transport system activity measurement, ETS). The original protocol was developed and described by De Coen and Janssen (1997). For this experiment, the protocol was adapted for use on digestive glands of *Mytilus edulis*. For each analysis, half a digestive gland was used (approx. 100 mg of wet weight). Total protein content was determined using the Bradford assay (Bradford, 1976).

Total carbohydrate content

The frozen digestive glands were allowed to thaw slowly on ice. After thawing, 100 μL of distilled water was added followed by homogenization. Afterwards, another 100 μL of deionized water was added. Subsequently, 100 μL of 15% trichloric adic (TCA) was added, the samples were mixed and left on ice. After ten minutes, the samples were centrifuged for 10 minutes at 4 °C and 1.500g. The supernatant was transferred to a new recipient and the pellet was resuspended in 200μL of 5% TCA. The centrifugation step was repeated and the resulting supernatant was added to the former followed by mixing. 250 μL of the supernatant was transferred to a new recipient after which 250 μL of 5% phenol and 1 mL of concentrated H_2SO_4 was added and the samples were mixed. The resulting extracts had to be diluted 1/2 using deionized water. Three replicates of 300 μL of each sample were transferred to a multiwell plate which was incubated in the dark. After 15 minutes, the optical density was determined at 495

nm. Glucose was used as a standard.

Total lipid content

The frozen digestive glands were allowed to thaw slowly on ice. After thawing, 100 μL of distilled water was added followed by homogenization. Afterwards, another 100 μL of deionized water was added. Subsequently, 500 μL of $CHCl_3$, 500μL of CH_3OH and 250 μL of deionized water were added, followed by mixing and centrifugation at 1.000g and 4 °C for 10 minutes. The top fluid phase was discarded. Digestive gland extracts were diluted 1/2 by adding 300μL of $CHCl_3$ to 300 μL of the bottom layer of the centrifugate. Three replicates of 100 μL were taken from each dilution and transferred to a 5 mL glass tube. These were dried at 60 °C for 30 minutes, followed by the addition of 500μL of H_2SO_4 and mixing. Subsequently, the samples were put in an oven at 200 °C for 15 minutes after which they were allowed to cool down to room temperature. When cooled down, 1.5 mL of deionized water was added to each sample followed by mixing. From each sample, 3 replicates of 250 μL were transferred to a multiwell plate. The optical density was measured at 340 nm. Tripalmitin was used as a standard.

ETS-activity

The frozen digestive glands were allowed to thaw slowly on ice. After thawing, the glands were homogenized on ice in 100 μL of a homogenate buffer at pH 8.5 containing 0.01 M PO_4, 0.05 M Tris-HCl, 75 μM $MgSO_4$, 1.5 mg mL^{-1} polyvinylpyrrolidone (PVP) and 2 mL L^{-1} Triton X-100. After homogenization, 300 μL of the homogenate buffer was added and the sample was mixed followed by centrifugation at 1.000g and 4 °C for 10 minutes. Three replicates of 60μL of supernatant were transferred to a multiwell plate. To each replica, 180 μL of a buffered substrate solution containing 0.01 M PO_4, 0.05 M Tris-HCl, 2 mL L^{-1} Triton X-100, 1.7 mM NADH and 0.25 mM NADPH. Subsequently, 60 μL of a 2 g L^{-1} INT solution was added to each well. The kinetic reaction was measured every 7 seconds at 490 nm.

4.2.3.3 ALP and lysosomal stability

The ALP and lysosomal stability biomarkers will be analysed in the second phase of the project. The suitable organs have been dissected, frozen in liquid nitrogen and subsequently stored at -80°C.

4.2.4 Mixture toxicity testing with passive samplers

4.2.4.1 Development

A new application under development in this project, aims to use passive samplers to administer chemical compounds to ecotoxicological test medium. In this way, environmental mixtures previously absorbed by these passive samplers in the field, are recreated in the laboratory. Also, as these passive samplers can replenish the amount of compound lost through processes like

evaporation, degradation and adsorption to test vessel walls, concentrations are expected to be constant in time.

To study this, experiments were conducted to investigate the equilibrium dynamics of the passive samplers. Spiked passive samplers were allowed to release their chemical load (fluorene, fluoranthene and benzo(a)pyrene) into uncontaminated water. The water concentrations were monitored to study concentration trends in time.

Additional modelling has been performed in an attempt to elucidate the mechanisms and functioning of the passive samplers. The release dynamics can be described by a one-compartment model (with the assumption that mass transfer in the aqueous phase is rate-limiting):

$$C_w = C_s \cdot Kd \cdot \left(1 - e^{-K_e \cdot t}\right) \quad (1)$$

$$K_e = \frac{D_w \cdot A}{\delta_w \cdot V_w} \quad (2)$$

where C_w is the water concentration ($\mu g.L^{-1}$), C_s is the concentration in the sampler ($\mu g.kg^{-1}$), Kd is the sampler-water partition coefficient ($L.kg^{-1}$), K_e is the system- and chemical-specific elimination rate constant (h^{-1}) and t is time (h).
This modeling was done for benzo(a)pyrene, fluoranthene and fluorene. These compounds have a diffusion coefficient in water (D_w) of $5.80 \cdot 10^{-6}$, $7.88 \cdot 10^{-6}$ and $6.35 \cdot 10^{-6}$ $cm^2\ s^{-1}$ respectively. To further determine K_e, a transport surface area A of 8 cm^2, an aqueous boundary layer thickness (δ_w) of 50 μm and an aqueous phase volume (V_w) of 0.05 L were used.

4.2.4.2 Mixture ecotoxicity testing

To conduct ecotoxicity tests with mixtures desorbed from contaminated passive samplers, the samplers (previously deployed at the stations shown in Figures 1 and 2) were transferred to 50 mL of uncontaminated test medium in a 100 mL erlenmeyer flask. Prior to addition of the test organism, the erlenmeyers were shaken for at least 1 hour on a rotary shaker to improve contaminant release from the samplers into the test medium. After one hour, equilibrium was assumed and the test organisms were added.

4.2.5 Quantitative Toxicological Interpretation and QSAR

The quantitative toxicological interpretation (QTI) approach was first used by Widdows *et al.* (1995). This approach uses QSARs to estimate the toxicity of the pollutants present in a mixture to a specific test organism. Based on these toxicity values, the measured water (or tissue) concentrations of the pollutants and the observed response of the test organism to the mixture, it is attempted at identifying the cause(s) of the observed effects.

In a first phase, the ECOSAR program of US EPA (Meylan & Howard, 1998) was used to calculate 96h growth inhibition EC50 values for algae of all the compounds analysed in this project, to make a first estimation of toxicity to marine phytoplankton and explore the possibilities of the proposed methodology. For each compound, the toxic units (TUs) present at each sampling station were calculated by dividing the measured water concentration (C_w) by its EC50 value:

$$TU = \frac{C_w}{EC50} \quad (3)$$

Additivity was assumed (Widdows *et al.*, 1995) and the sum of TUs was calculated per station. The results were compared to the results of the growth inhibition test with *P. tricornutum*.

As the endpoint calculated by ECOSAR was one for freshwater algal species, literature data were gathered for marine diatoms; the same was done for bivalve larvae. A clear lack of ecotoxicity data for marine species became apparent during this literature survey. QSARs could be developed for phenols, PAHs and N-pesticides for both marine diatoms and bivalve larvae. However, these should be considered as very rudimentary due to the lack of available literature data. The QSARs were used to conduct a first QTI of the data from the tests with *P. tricornutum* and *C. gigas* larvae using the approach described above.

In this first stage water concentration data were used as no data were yet available on contaminant concentrations in the passive samplers. For the EC50, literature data was used. Additivity was assumed to determine the sum of TUs of the mixtures. Other models can and will be explored in the second phase of the project.
The QTI approach has been used to interpret results of ecotoxicological tests with *P. tricornutum* and *C. gigas* exposed to mixtures desorbed from contaminated passive samplers in the 72h growth inhibition and the 24h larval development tests respectively (see 5.2.1 and 5.2.2).

4.3 Ecological monitoring

4.3.1 Benthic fauna monitoring

During Belgica / Zeeleeuw / Zeekat sampling campaigns macrobenthos, hyperbenthos and epibenthos were collected. Epibenthos was sampled on the stations W01 - W06 and S01, with a 2.7 m beam trawl with mesh size of 6 mm. The position of the trawling stations is slightly different from the standard stations as the sediment composition of the bottom was not always appropriate. Trawling was done with the current for 1000 m at an average ship speed of 4.5 knots. All epibenthic biota were sorted out on board, identified to species level, counted and fish were measured. Liver of flatfishes (*Limanda limanda*, *Solea solea* and *Pleuronectes platessa*) and brown shrimps (*Crangon crangon*) were collected for chemical analyses. Hyperbenthos was sampled by means of the hyperbenthic Sorbe-sledge (Sorbe, 1983) on the Belgica. On the Zeeleeuw, hyperbenthos was quantitatively sampled by means of a small hyperbenthic sledge because the Sorbe-sledge can not be deployed on board the Zeeleeuw. Trawling was always done against the current over an average distance of 1000 m at an average ship speed of 4.5 knots. A current meter was always deployed. On each sampling location the samples of the 1 mm upper and the samples of the 1 mm lower net were preserved in a buffered formaldehyde solution of 7%. In the harbours hyperbenthos was sampled by a net with a mesh size of 1 mm and a diameter of 0.57 m. Macrobenthos was sampled using a Van Veen grab of 0.1 m² surface area on the stations S22, S01 and W01 – W06. In the harbours a smaller, 0.0314 m² surface area, manual Van Veen grab was used. At each station 3 replicas were taken. The samples were

sieved through a 1 mm mesh-sized sieve and preserved in a buffered formaldehyde solution of 7%. Sediment for granulometric analyses was sampled at each station.

4.3.2 Blue mussel and pacific oyster cage experiments

Two cage experiments were conducted during the first phase of the project. A first experiment ran from June till October '07. Three stations were sampled: outport (ZB3) and the marina of Zeebrugge (ZB2), and the Sluice dock of Oostende (OO1), representing a gradient from the inner harbour to the outer harbour. A second experiment was set up from February till July '08. Four stations were sampled: the marina of Zeebrugge (ZB2), the marina of Oostende (OO2), the marina of Nieuwpoort (NP2') and a station situated at sea on the Nieuwpoortbank (W03').

Mussels and oysters were collected in the Eastern Scheldt from the subtidal mussel plots and were transplanted to cages deployed at the different stations. At each station two cages are used as replicas. The organisms were sampled monthly to determine growth, condition index, gonad development and body concentrations of micropollutants.

Growth is determined by Shell Length Increment and calculated from following equation: SLI (μm/day) = $(SL_{t+1} - S_t)$ / dt (Jantz & Neumann, 1998). Shell length (i.e. the maximum dimension along the anterior-posterior shell axis) was measured to the nearest 0.01 mm using an electronic vernier calliper.

To determine their condition index; mussels were sampled monthly and oysters (bi)monthly. Total wet weight, wet weight of the soft tissue and shell and the shell length were measured. Tissue and shell were oven dried for at least 48 h at 60 °C to obtain dry weight. Two condition indices were calculated. CI_1 = (dw tissue (g) x 1000) / shell length (mm) after Bayne & Widdows (1978). CI_2 = (dw tissue (g) x 100) / dw shell (g) after Walne & Mann (1975).

Mussels and oysters (same sampling frequency as above) were collected to study gonad development. In the laboratory, gonads were removed, fixated in Bouin's fluid and stored in 70 % ethanol. The tissues were embedded in paraffin (60 °C) and sections cut at 5 – 10 μm were stained with toluidin blue (Pearse, 1985). Gametogenic development was determined according to Seed (1975). Individual length (mm) was also determined.

Water temperature (°C), salinity (PSU), oxygen content ($mg.L^{-1}$), chlorophyll a concentration ($\mu g.L^{-1}$) and suspended particulate matter (SPM) ($mg.L^{-1}$) were monitored fortnightly. Salinity and temperature were measured by a Conductivity Meter LF320, while oxygen content was measured by a Dissolved Oxygen Meter YSI 52. Chlorophyll a concentration was measured in a 1000 ml sample filtered through a Whatman GF/F filter which was analysed with a HPLC-sampler according to Jeffrey *et al*. (1997).

4.3.3 Tern egg collection

Subcontractant INBO sampled ten eggs of *Sterna hirundo* in the tern breeding colony in the harbour of Zeebrugge during spring 2007 and spring 2008.

4.4 Preliminary integrated risk assessment

Environmental and toxicological risk characterisation will be performed according to the REACH (Registration, Evaluation and Authorisation of Chemicals) Implementation Project (RIP) 3.2.2 Part E: Risk Characterisation. RIPs are guidance documents for the practical implementation of the REACH regulation that entered into force on 1st June 2007 to streamline and improve the former legislative framework on chemicals of the European Union (EU).

In risk characterisation, exposure levels are compared to quantitative or qualitative hazard information. Risk characterisation ratios (RCRs) are derived by comparing exposure levels (PECs - Predicted Environmental Concentrations) to suitable Predicted No Effect Concentrations (PNECs) or derived no-effect levels (DNELs) for respectively environmental and human health endpoints. If the PEC value is situated below the PNEC or the exposure level is lower than the DNEL, the risk is adequately controlled. If the PEC exceeds PNEC or if the exposure level is higher than the DNEL, the risk is not controlled.

Data on physico-chemical parameters, toxicological dose descriptors and ecotoxicological endpoints were gathered through different sources:

- ECB-ESIS (European Chemicals Bureau - European Chemical Substance Information System) website: http://ecb.jrc.it/esis
- EU-RAR of ARCADIS Belgium (former EURAS)
- ATSDR (Agency of Toxic Substances & Disease Registry) website: http://www.atsdr.cdc.gov/toxpro2.html
- Footprint Pesticide Properties Database: http://sitem.herts.ac.uk/aeru/footprint/en/atoz.htm
- Scientific literature through Web of Science

5 Results

5.1 Exposure assessment

5.1.1 Conventional chemical analysis

5.1.1.1 Organotins

In sediment, concentrations of TBT up to 80 μg.kg^{-1} dry weight were measured. Stations S22, OO1, ZB01 and ZB02 showed highest concentrations. $\Sigma_6$organotins (MBT, DBT, TBT, MPhT, DPhT, TPhT) gave concentrations of up to 140 μg.kg^{-1} dry weight. TBT/DBT ratio's varied from 0.7 to 4, with lower ratio's indicating a more pronounced degradation of TBT into DBT. This was particularly the case for the Scheldt estuary and in some samples from the open sea, while degradation in harbours was less pronounced.

TBT concentrations in the water phase varied from < 0.05 to 11.9 ng.L^{-1} and for DBT from < 0.05 to 5.5 ng.L^{-1}. Concentrations of the other organotin compounds were lower than 0.8 ng.L^{-1}. The highest concentrations for TBT were found at stations W01, NP03, ZB01 and S22. The TBT contribution to the total amount of organotin varied from 16 to 79%. A higher TBT contribution indicates more recent input. This was particularly the case for the harbours of Zeebrugge and Nieuwpoort. Concentrations of $\Sigma_6$organotins of water and sediment were put in a Box and Whisker plot (Figure 3).

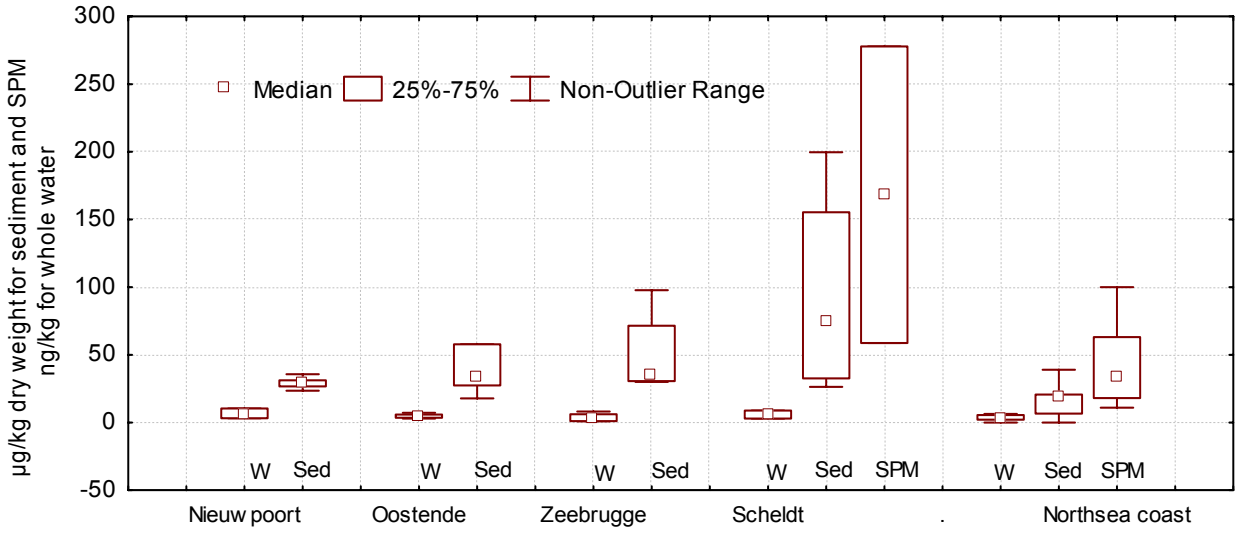

Figure 3. Box and Whisker plot for $\Sigma_6$organotins grouped in 5 sampling regions, Nieuwpoort harbour, Oostende harbour, Zeebrugge harbour, the Scheldt Estuary and the Northsea coast in the water phase (W), Sediment (Sed) and Suspended matter (SPM).

5.1.1.2 PAHs

PAH concentrations measured in sediment were highest for the compound fluoranthene (up to 1000 μg.kg^{-1} dry weight), followed by benzo(b)fluoranthene and pyrene, with maximum concentrations of 300 and 460 μg.kg^{-1} dry weight respectively. In general, sediment of the stations

S22, OO1 en OO2 were most polluted with PAHs with concentrations for Σ_{22}PAHs of up to 3000 μg.kg^{-1} dry weight. Highest PAH concentrations were found in sediment samples from NP2 collected during the summer.

Concentrations of Σ_{22}PAHs varied from 16 to 350 ng.L^{-1}. The harbours of Antwerp, Oostende and Zeebrugge were the most polluted of all samples areas. The lowest concentrations were measured during the summer period in open sea. Napthalene, 2,6-dimethylnaphthalene, benzo(b)- and benzo(k)fluoranthene were the most abundant, with concentrations up to 75 ng.L^{-1}. Concentrations of Σ_{22}PAHs of water and sediment are to be viewed in a Box and Whisker plot (Figure 4).

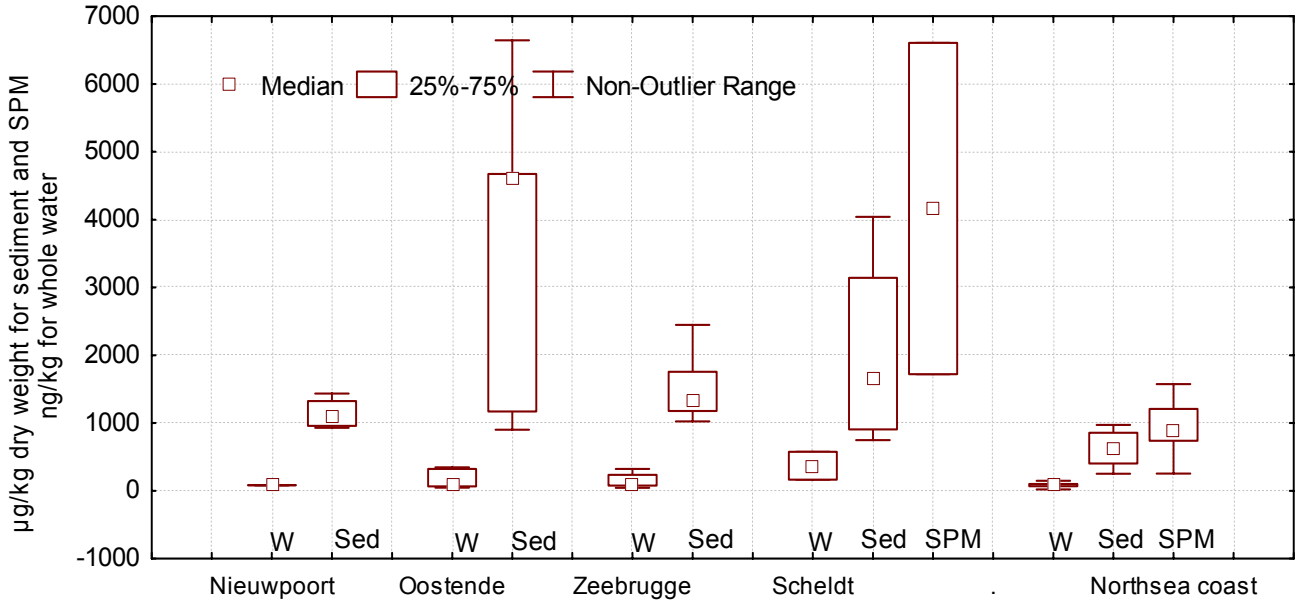

Figure 4. Box and Whisker plot for Σ_{22}PAHs grouped in 5 sampling regions, Nieuwpoort harbour, Oostende harbour, Zeebrugge harbour, the Scheldt Estuary and the Northsea coast in the water phase (W), Sediment (Sed) and Suspended matter (SPM).

5.1.1.3 BFRs

Over 98% of total PBDE-content in sediment consisted of the BDE-209 congener. BDE-209 is the most widely produced formulation. Concentrations in our study varied from $<$0.1 to 800 μg.kg^{-1} dry weight in sediment and from $<$0.1 to 950 μg.kg^{-1} dry weight in SPM. These concentrations are similar to those found in other studies. The highest concentrations were found at S22 and ZB03, the lowest concentrations for the open sea stations. Σ_6BDEs (BDE-28, -47, -99, -100, -153 and -154) varied from 0.03 to 5.63 μg.kg^{-1} dry weight in sediment. Highest concentrations were found in SPM on W02 and S22 of respectively 9 and 10 μg.kg^{-1} dry weight. After BDE-209, congeners BDE-47 and BDE-99 were most abundant.

Concentrations Σ_6BDEs varied from $<$ 0.05 to 20 ng.L^{-1} in the water phase. The Σ_6BDEs differed significantly (t-test, P = 0.05) between samples collected during the summer campaign 2007 (range 0.11 to 1.34 ng.L^{-1}, median: 0.33 ng.L^{-1}) and those from the winter campaign 2007 (range 0.19 to 20 ng.L^{-1}, median: 2.47 ng.L^{-1}), with higher concentrations in winter period. Reasons for this are yet

unclear but not related to the content of suspended matter or TOC. Concentrations of Σ_6BDEs of water and sediment are presented in a Box and Whisker plot (Figure 5).

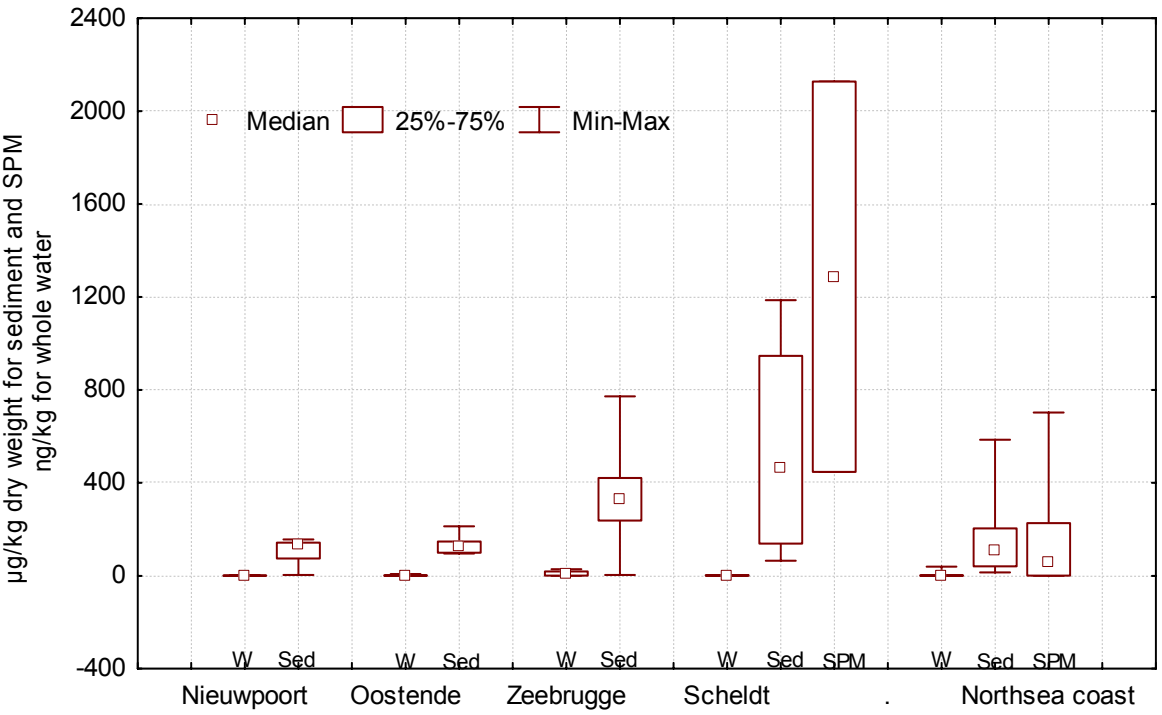

Figure 5. Box and Whisker plot for Σ_6BDEs grouped in 5 sampling regions, Nieuwpoort harbour, Oostende harbour, Zeebrugge harbour, the Scheldt Estuary and the Northsea coast in the water phase (W), Sediment (Sed) and Suspended matter (SPM).

5.1.1.4 Phenols

The occurrence in the Belgian coastal zone of octylphenol and nonylphenol was studied within INRAM. According to the Water Framework Directive (Council Directive 2000/60/EC) an EQS of 0.01 and 0.3 μg.L^{-1} was set for octylphenol and nonylphenol respectively. All concentrations of octylphenol were lower then the LOD (= 0.015 μg.L^{-1}). Figure 6 shows the detected concentrations of nonylphenol in the water samples of the different sampling campaigns (May 2007, December 2007 and April 2008.

Nonylphenol was detected at several sampling locations of the May campaign. The highest levels were found at S22 (Scheldt estuary in Antwerp), OO4 (harbour of Ostend) and ZB4 (harbour of Zeebrugge) in concentrations varying from 0.8 to 1.1 μg.L^{-1}. At these stations the EQS was exceeded. In December and April, the measured concentrations of nonylphenol were below the EQS. Obviously, nonylphenol was hardly detected in the open sea. However, nonylphenol was found in a concentration of 0.50 – 0.60 μg.L^{-1} at W06. The analytical approach included several other phenolic compounds. Dettol, 4-chloor-3-methylfenol and bisfenolA were regularly detected. Maximum concentrations of 0.06, 0.21 and 0.180 μg.L^{-1}, respectively, were found. Especially bisfenolA is interesting, because this compound is a known endocrine disruptor (Toppari et al., 1996).

Priority was given to analysis of water samples. Currently new procedures are being developed to determine phenols in sediment and biota.

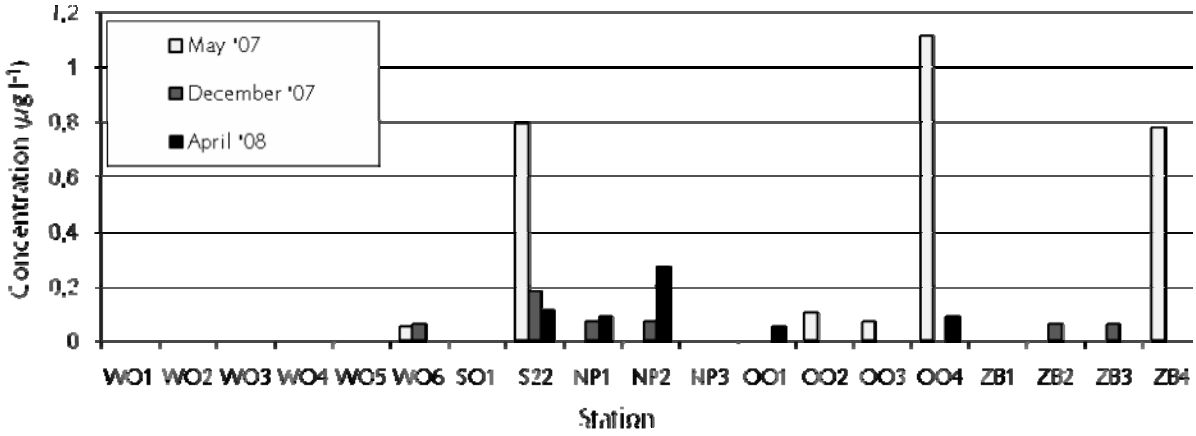

Figure 6. Concentrations of nonylphenol in the water column at the different sampling locations for the three sampling campaigns (LOD = 0.05 μg.L^{-1}).

5.1.1.5 Organonitrogen pesticides

The solubility in water, half life in soil and mobility in surface and ground water enable organonitrogen pesticides (ONPs) to reach estuarine areas (Gascón et al., 1998). Therefore, the presence of ONPs in the Belgian coastal zone was studied within INRAM. The accent concerning the ONPs was set on alachlor, atrazine, diuron, isoproturon, simazine and terbutylazine. The water samples of the three campaigns (May 2007, December 2007 an April 2008) were analysed. According to the Water Framework Directive (Council Directive 2000/60/EC), environmental quality standards (EQSs) were established for five of the selected ONPs: alachlor, atrazine, diuron, isoproturon and simazine (See Table 1). For terbutylazine a PNEC-value (PNEC = Predicted No Effect Concentration) was used, because so far no EQS was established for this compound (Flemish Environmental Agency, De Wulf E., Personal Communication). The maximum detected concentrations and the concentrations exceeding the EQSs (or PNEC) of the targeted ONPs are shown in table 1.

Alachlor and atrazine were particularly detected in the Scheldt Estuary (SO1 and S22) and in the harbour of Ostend and Nieuwpoort. Detected concentrations were respectively up to 0.083 μg.L^{-1} and 0.077 μg.L^{-1}. Diuron was frequently detected, especially in the samples of the December campaign. Levels up to 0.454 μg.L^{-1} (NP1, harbour of Nieuwpoort) were measured. For diuron the EQS was exceeded at sampling locations NP1, S22 (Scheldt estuary in Antwerp) and OO2 (harbour of Ostend). Isoproturon was detected at most sampling locations in December 2007 as well as in April 2008. The highest concentrations were found at S22, in the harbour of Nieuwpoort and Ostend (0.2 – 0.3 μg.L^{-1}). Lower concentrations of isoproturon (< 0.05 μg.L^{-1}) were measured at the offshore sampling locations. Simazine was found in most harbour-samples. Measured concentrations were up to 0.060 μg.L^{-1}. For terbutylazine the highest concentrations (± 0.35 μg.L^{-1}) were found in the Scheldt estuary near Antwerp (S22) and in the harbour of Ostend (OO2) in April 2008. At these sampling locations, the considered PNEC-value was exceeded. Generally, the targeted ONPs were detected more frequently and in higher concentrations in the harbour sampling points in comparison with the sampling points at

open sea. The analytical method included the determination of a wide spectrum of ONPs. Several other ONPs were frequently detected and need obviously also attention: 2-hydroxy-atrazine, chlortoluron, metolachlor and propachlor were detected in significant amounts at several sampling locations.

Table 1. Maximum detected concentration and concentrations exceeding the Environmental Quality Standards (EQS) of the ONPs of interest at the different sample stations (LOD = Limit of Detection). For terbutylazine a PNEC-value was used, because no EQS was established for this compound (*).

	Maximum detected concentration		**Concentrations > EQS**		**LOD**	**EQS**
Compound	Station	$\mu g.L^{-1}$	Station	$\mu g.L^{-1}$	$\mu g.L^{-1}$	$\mu g.L^{-1}$
Alachlor	S01	0,083	-	-	0.006	0,3
Atrazine	S22	0,077	-	-	0.003	0,6
Diuron	NP1	0,454	NP1	0,454	0.006	0,2
			S22	0,254		
			OO2	0,35		
Isoproturon	NP1	0,292	-	-	0.003	0,3
Simazine	ZB3	0,06	-	-	0.003	1
Terbutylazine	S22	0,347	S22	0,347	0.003	0.23*
			OO2	0,326		

5.1.1.6 Pharmaceuticals

An analytical method was developed that enables the detection of 15 pharmaceuticals in surface water, including sea- and freshwater. The list of targeted analytes included four antibiotics (sulfamethoxazole, ofloxacin, trimethoprim, chloramphenicol), five non-steroidal anti-inflammatory drugs (NSAIDs) (mefenamic acid, diclofenac, salicylic acid, paracetamol, ketoprofen), two β-blockers (propranolol, atenolol), two lipid regulators (bezafibrate, clofibric acid), one psychiatric drug (carbamazepine) and one β-agonist (salbutamol). The analytical approach consists of extraction of the pharmaceuticals by solid phase extraction (SPE) and identification by liquid chromatography tandem mass spectrometry (LC-MS). SPE combining LC-MS is currently the common used technique to obtain accurate and sensitive analysis of pharmaceuticals in water samples (Gómez et al., 2006; Nebot et al., 2007; Ternes et al., 2001; Hernando et al., 2006; Gros et al., 2006). An extensive validation study of the new developed method was carried out according to Commission Decision 2002/657/EC. The obtained validation parameters (not reported) demonstrated the applicability of the analytical approach. Except for paracetamol and salbutamol, all targeted pharmaceuticals can be determined quantitatively. The limits of quantification (LOQs) vary between 0.001 and 0.1 $\mu g.L^{-1}$, depending on the compound.

The developed method was applied to the collected water samples within INRAM (sea 4.1.). Two antibiotics were detected in the three sampling campaigns (May 2007, December 2007 and April 2008). Trimethoprim was found once in each campaign in a concentration up to 0.029 $\mu g.L^{-1}$. Concentrations of sulfamethoxazole were < LOQ (= 0.010 $\mu g.L^{-1}$) to 0.096 $\mu g.L^{-1}$. Both antibiotics were detected in the harbours of Ostend and Nieuwpoort, while sulfamethoxazole was also measured twice in the Scheldt estuary in Antwerp. Concentrations of other antibiotics

were below the established LOQs. The widely used NSAID paracetamol could only be determined qualitatively and was found in all samples considered. Salicylic acid, the deacylated more active form of acetylsalicylic acid, was detected in a concentration of ± 0.100 $\mu g.L^{-1}$ at most sampling locations. However higher concentrations, up to 0.855 $\mu g.L^{-1}$, were found in the harbours of Zeebrugge and Ostend, in the Scheldt estuary in Antwerp and at the offshore locations W01 and W02. β-blockers atenolol and propranolol were only found at the harbour stations and in the Scheldt in Antwerp. For atenolol concentrations varied from < LOQ to 0.293 $\mu g.L^{-1}$, while concentrations of propranolol were limited to 0.024 $\mu g.L^{-1}$. Concentrations of bezafibrate were limited to 0.018 $\mu g.L^{-1}$. The psychiatric drug carbamazepine was very often detected in levels up to 0.321 $\mu g.L^{-1}$. In general, the Scheldt in Antwerp and the harbour stations (Zeebrugge, Nieuwpoort, Ostend) were most polluted with pharmaceuticals, irrespective of the sampling period.

The method development and validation study were performed in the first year and a half of the project. Priority was given to analysis of water samples. Currently new procedures are being developed to determine pharmaceuticals in sediment and biota in the low $ng.L^{-1}$ level.

5.1.1.7 Phthalates

Because the risk for contamination was too high, the samples of 2007 were not analysed. In cooperation with the FEA, the sampling procedure for phtalates was optimized to obtain uncontaminated water samples. The water samples of the sampling campaign of April 2008 are currently being analysed.

5.1.1.8 Perfluorinated compounds

The development of an analytical approach was finished. Water samples of the sampling campaigns of the last two years of the project will be analysed.

5.1.2 Passive sampling

PAHs were measured in passive samplers, deployed in 2007 and 2008. The samplers were exposed during a period of 43 to 110 days, depending on the sampler site. Fluorene-D10, phenanthrene-D10 and benzo(e)pyrene-D12 were used as PRCs and gave information about the sampler kinetics during the deployment. Sampling rates, calculated from the dissipation of the PRCs varied from 1.3 $L.d^{-1}$ to 126 $L.d^{-1}$. Sites with less turbulence, such as OO1 (enclosed site), showed significantly lower sampling rates, compared to sites with a lot of turbulence, like open sea sites (A2 and R&V). Generally equilibrium was reached up to compounds with Log K_{ow} < 5. For compounds with higher hydrophobicity the uptake kinetics were in the linear uptake phase. Concentrations in the water phase of Σ_{22}PAHs are shown in figure 7. Passive samplers measures PAHs freely dissolved in the water phase and not PAHs bound to suspended particles and humic acids.

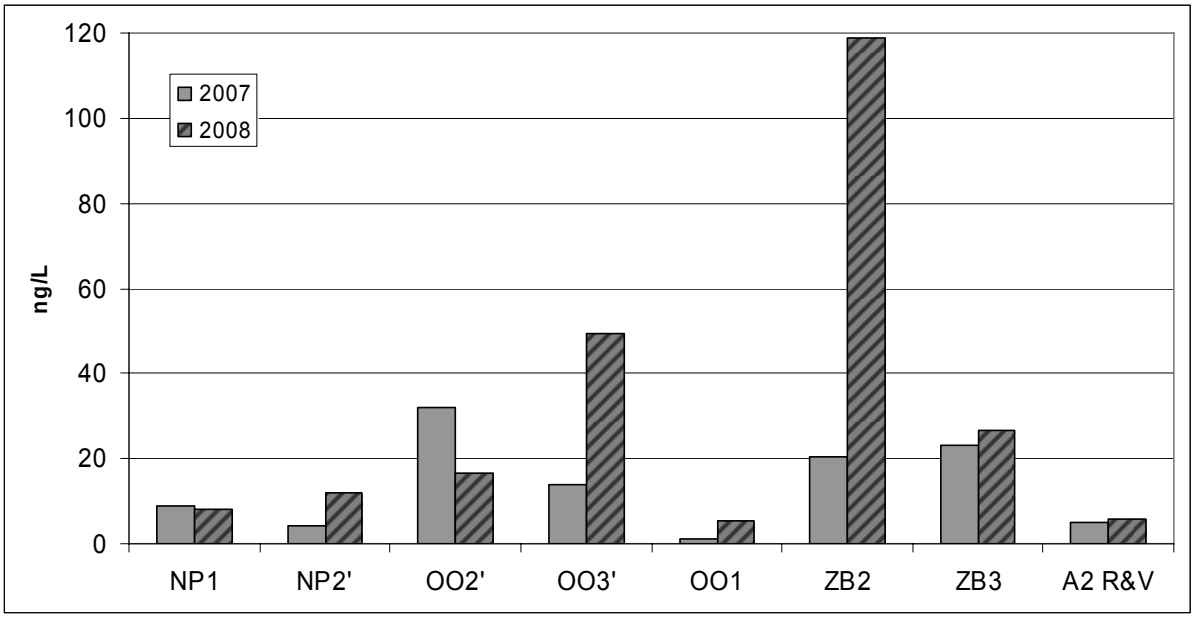

Figure 7. Concentrations of Σ_{22}PAHs in the water phase measured with passive samplers during 2007 and 2008.

Passive samplers estimate the concentration of PAHs that is freely dissolved in the water phase and not bound to suspended particles and humic acids, also known as total water concentrations. Therefore more soluble PAHs (low K_{ow}) were found in higher concentrations (up to 67 ng.L^{-1}) than less soluble PAHs (0.001 to 0.020 ng.L^{-1}). Generally Nieuwpoort harbour showed lowest contamination with PAHs. This corroborates with the measurements in total water and sediment. Oostende and Zeebrugge showed highest concentrations, with Σ_{22}PAHs varying from 14.02 to 118.96. The contaminants fluorene, 2,6-dimethylnaphthalene and acenaphthene were most abundant. This indicates contamination of petrogenic origin, possibly from diesel fueled vehicles.

5.2 Effect and bioaccumulation assessment

5.2.1 Biomarker experiments

5.2.1.1 CEA

Results of the CEA biomarker show reduced energy reserves of *M. edulis* in the harbour of Nieuwpoort (see Figure 8). Lipid, carbohydrate and protein levels in this harbour were all significantly lower than control values. This could be due to the high accumulation of sediment in the cages deployed in Nieuwpoort. Since most of the organisms there were entirely buried, the resulting inability to feed is the most likely cause of the significant reduction of energy levels observed at this station.

A significant reduction of carbohydrate levels was observed in Oostende harbour. Lipid content was reduced significantly in Zeebrugge harbour at both stations. No sediment accumulation was observed at these locations.

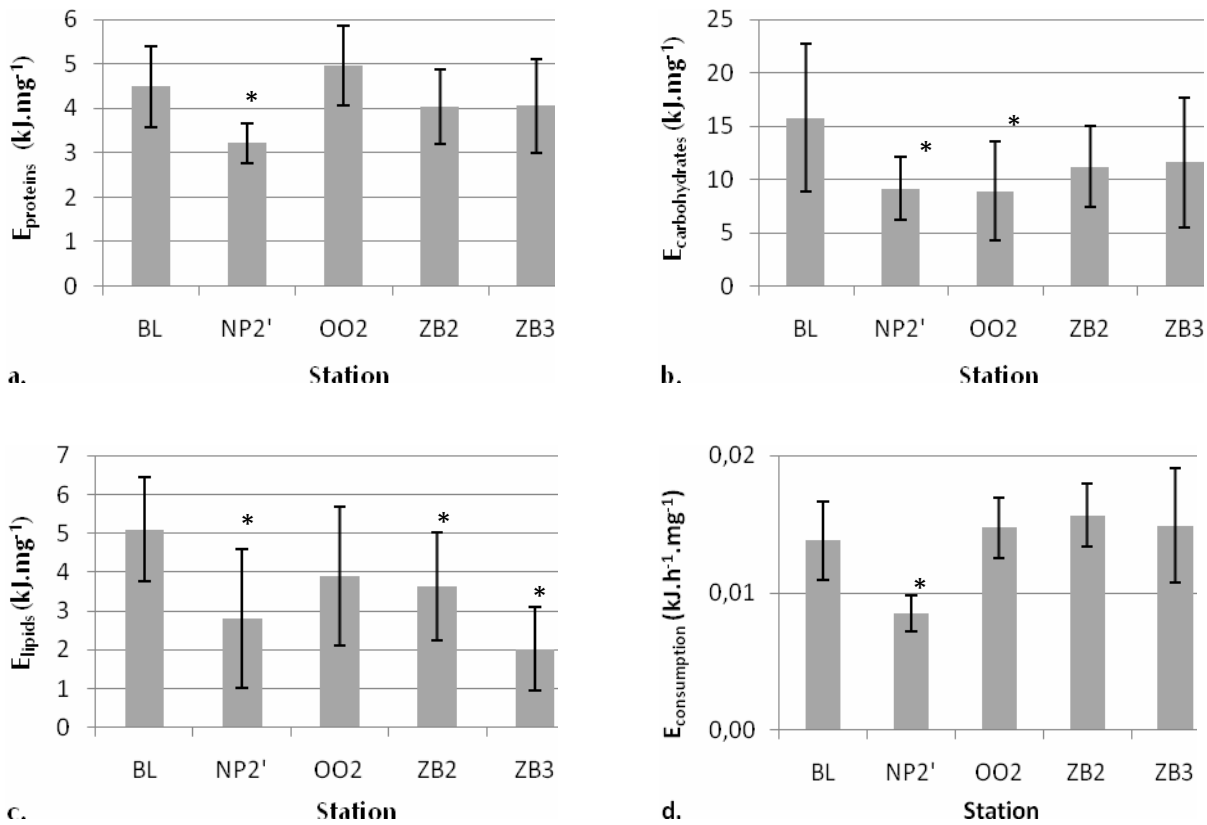

Figure 8. Results of the cellular energy allocation analysis of mussel digestive glands after *in situ* exposure to harbour waters.(+/- SD) *: significantly lower than control.

The organisms deployed at the station in Nieuwpoort showed a reduced metabolic activity, as they exhibited a significantly lower ETS activity than the control organisms. This could also be due to the high sediment accumulation. Overall, this caused the CEA parameter to be equal for the organisms deployed in Nieuwpoort (1793.5 ± 685.8) and the control organisms (1847.9 ± 650.8).

No significant difference in ETS activity was observed at the station in Oostende or the stations in Zeebrugge. Overall CEA appeared to be lower at these stations (OO2: 1206.1 ± 385.1; ZB2: 1206.7 ± 413.6; ZB3: 1190.1± 503.9).

5.2.1.2 EROD

The analysis of the EROD biomarker shows a significant increase of EROD activity in all harbours (see Figure 9). In the harbour of Zeebrugge, however, the station located in the inner harbour – station ZB2 – does not show a significant increase. This could be due to either very low pollution by EROD inducing compounds, or to a much higher pollution of these contaminants at this station, as the EROD biomarker is known to have a bell-shaped response curve. Chemical analysis of EROD inducing compounds in mussel tissue and passive samplers deployed simultaneously with the cage experiment, will help to clarify the results of this biomarker.

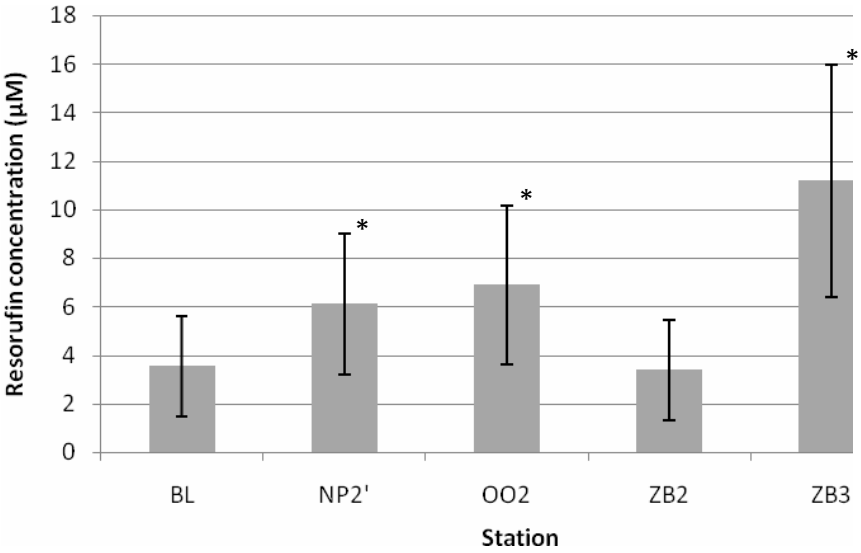

Figure 9. Results of the EROD analysis of mussel digestive glands after *in situ* exposure to harbour waters.(+/- SD) *: significantly higher than control.

5.2.2 Mixture toxicity tests

5.2.2.1 Development

Results of the modeling experiments (see 5.2.4.1) indicate that, for 1 g of sampler added to 0.05L of water, compounds with a log(Kd) lower than 2.7 will be depleted by 10% in the sampler (increasing with decreasing log(Kd); see Figure 10a). If for example 10 g of sampler is added, this $\log(Kd)_{10\%}$ would be 1.7. As such, an optimal amount of sampler needs to be chosen in order to ensure that the sampler does not hamper the ecotoxicity test (e.g. by decreasing the incoming light during growth-inhibition tests with algae), and to recreate the mixture from the environment as good as possible.

Figure 10b shows the release kinetics of 1 g of sampler with a transport surface of 8 cm^2 for 3 compounds with varying log(Kd). An aqueous boundary layer thickness of 50 μm was assumed. Results showed that the time to reach 95% of the equilibrium concentration, $t_{95\%}$, varied from 3.3h for a log(Kd) of 3.8, to 4.5h for a log(Kd) of 5.6.

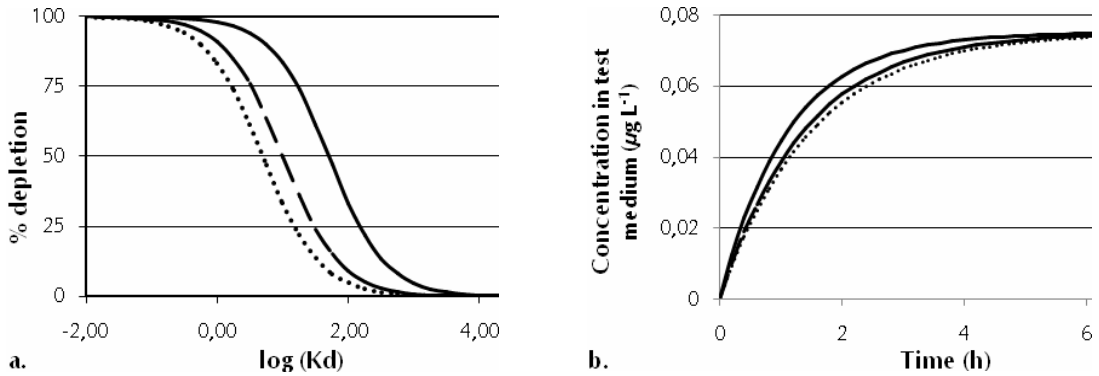

Figure 10. (**a**) Relation between % of depletion of the passive sampler and Log(Kd) of the absorbed compound, volume of the test medium was assumed to be 0.05L, the amount of sampler added was 1g (solid line), 5g (dashed line) or 10g (dotted line); (**b**) release kinetics of compounds with varying Log(Kd): fluorene (solid line; log(Kd) = 3.82), fluoranthene (dashed line; log(Kd) = 4.69), benzo(a)pyrene (dotted line; log(Kd) = 5.56).

5.2.2.2 Mixture ecotoxicity testing

Figure 11 shows the results of the 72h growth inhibition test with the marine diatom *P. tricornutum*. This test was repeated three times and an excellent reproducibility was found. Samples collected at the station at the Royal Yacht Club in the harbour of Oostende were the only ones for which an adverse effect was observed. Algae exposed to the mixtures from passive samplers previously deployed at this station, consistently showed a significant growth inhibition.

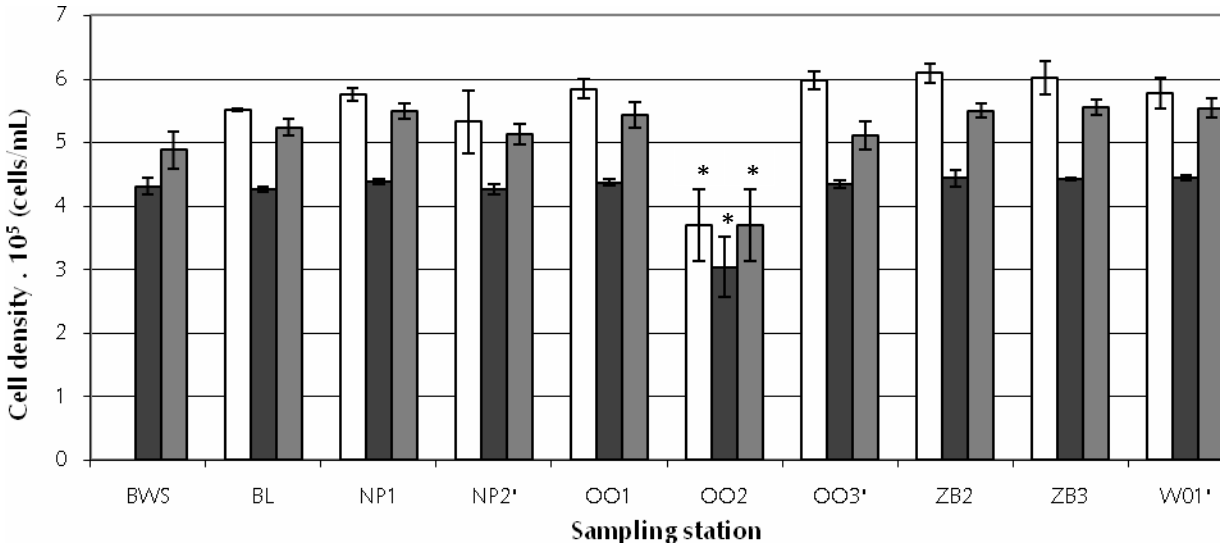

Figure 11. Cell density of *P. tricornutum* (+/- SD) exposed for 72h to mixtures desorbed from passive samplers. Each shading represents one test. BWS: blanks with sheets, BL: blanks without sheets. *: significant growth inhibition compared to control with blank sheets.

Other tests that were performed with (field deployed) passive sampler extracts were the 24h larval development test with *Crassostrea gigas* (see annual report of 2007) and a 7 day cellular

energy allocation assay with *Mytilus edulis*. The results are summarised in Table 2.

Table 2. Summary of the results of the ecotoxicological tests performed with mixtures desorbed from passive samplers (deployed: August – September 2007). The different shading indicates the gravity of the observed effects (☐: no effect; ▒: moderate effect; ■: severe effect; ▨: not (yet) determined). ETS: electron transport system activity in digestive gland; N: Normal.

	***C. gigas*: 24h larval development test**		***P. tricornutum* 72h growth inhibition test**	**7 day cellular energy allocation assay with *Mytilus edulis***			
Station	% abnormal	% dead	% growth inhibition	Proteins	Lipids	Carbohydrates	ETS
NP1	45%	6%	0	N	N	N	Elevated
NP2′	70%	17%	0	N	N	N	Elevated
OO1	70%	26%	0	N	N	N	N
OO2	0%	100%	12%	N	N	N	N
OO3′	30%	69%	0	Elevated	N	N	N
ZB2	17%	83%	0	N	Lowered	N	N
ZB3	65%	34%	0	N	Lowered	N	N
W01′			0	N	N	N	Elevated

5.2.3 Quantitative Toxicological interpretation

Station OO2, the only one exhibiting an effect on the diatom, also showed the highest value for the sum of TUs. Further analysis showed that nonylphenol and TBT accounted for roughly 33% of the total observed toxicity. 90% of the total observed toxicity could be explained by these two compounds combined with 9 PAHs and 1 pesticide (metolachlor). Using this approach, samples collected at other stations were identified that could potentially exhibit an effect on *P. tricornutum*. Stations OO4, ZB4 and S22 (stations at which no passive samplers had been deployed) all showed a higher sum of TUs than that calculated for station OO2 (OO2: 0.0065; OO4: 0.0256; ZB4: 0.0183; S22: 0.0215).

As the endpoint calculated by ECOSAR was one for freshwater algal species (6.2.2.2), literature data were gathered for marine diatoms; the same was done for bivalve larvae. A clear lack of ecotoxicity data for marine species became apparent during this literature survey. QSARs could be developed for phenols, PAHs and N-pesticides for both marine diatoms and bivalve larvae. However, these should be considered as very preliminary due to the lack of available literature data. The QSARs were used to conduct a first QTI of the data from the tests with *P. tricornutum* (Figure 12a) and *C. gigas* larvae (Figure 12b) using the approach described above. Figure 12a and 7b indicate the highest sum of TUs at station OO2 for both test species. This is in accordance with the results of the ecotoxicological tests performed with passive samplers and both these organisms (see Figure 11 and Table 2) which show the most severe effects at this station. It is also apparent that the toxicity to marine diatoms is mainly caused by phenols. For bivalve larvae, PAHs and TBT seem to be the most important compounds (figure 12b). As above, stations OO4, ZB4 and S22 have the potential to cause more severe toxic effects to both marine diatoms and bivalve larvae. Station OO4 approached a toxic unit of 1, even when not all

compounds were included in this calculation yet. Pharmaceuticals for example, were not included yet as effect data on these marine species was not available. However, it has been shown that for example antibiotics can have severe effects on marine diatoms. Ferrari *et al*. (2004) found a *Cyclotella meneghiniana* 96h growth inhibition EC50 of 90.6 μg.L^{-1} for the antibiotic ofloxacin.

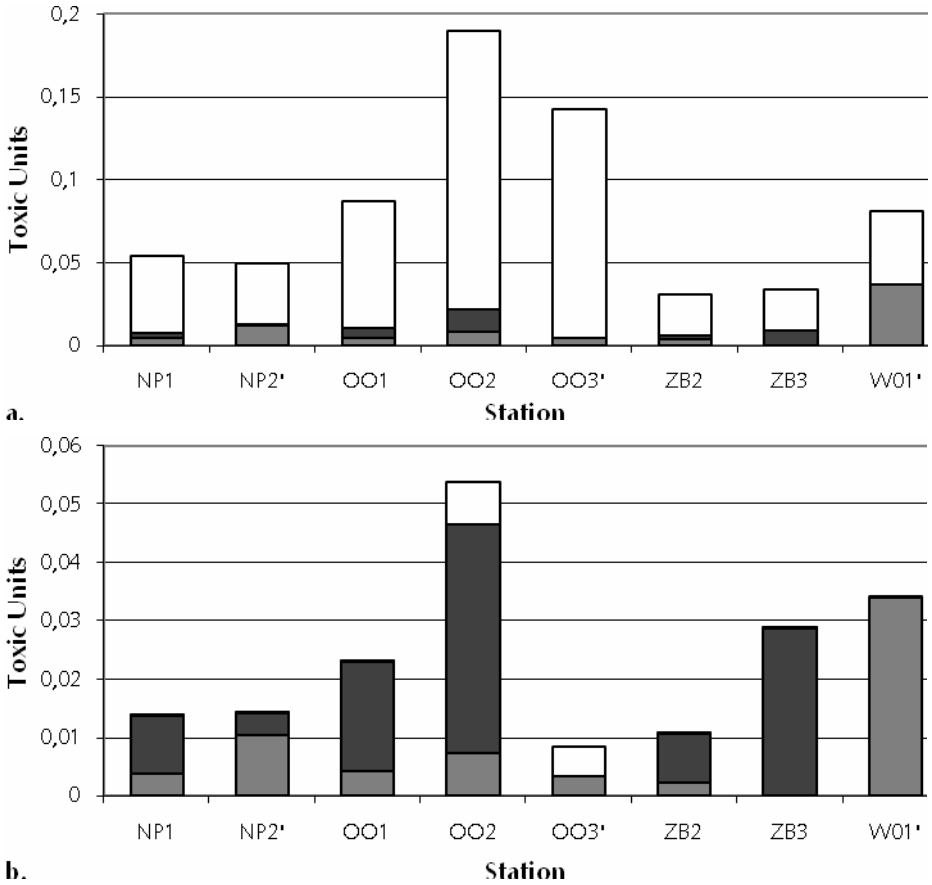

Figure 12. Sum of the toxic units at each station sampled with passive samplers, for (**a**) the 72h growth inhibition test with *P. tricornutum*, and (**b**) the 24h larval development test with *C. gigas*. □: phenols; ■: PAHs, ■: TBT; ■: N-pesticides.

5.3 Ecological monitoring

5.3.1 Cage-Experiment Jun - Oct '07 (focus on *Mytilus edulis*)

The first experiment was conducted at the outport (ZB3) and the marina of Zeebrugge (ZB2) and the Sluice dock of Oostende (OO1). In general, the results show a spatial gradient in shell length (SL), shell length increment (SLI) from outer harbour to inner harbour. The highest SL and SLI values are obtained in ZB3, followed by ZB2 while the weakest values were measured at OO1. The Condition Index (CI1) has a slightly different pattern as the highest values are measured in ZB3 first followed by OO1 and then by ZB2.

The biometric parameters (SL, SLI & CI) were statistically tested and they all showed a similar pattern. The Mann Whitney U-test indicates no significant difference in the variables between the two replicas at each station ($p > 0.05$). Kruskal Wallis analysis shows a significant difference between the stations ($p < 0.05$).

Although similar trends were found between temp, chla and the SL, SLI and CI, no significant correlations (non-parametric Spearman Rank correlation) could be found. Further data analysis, including the chemical analyses data, may reveal more patterns.

The study of the gametogenic development revealed that the mussels were in the transition between the spawning and spent stage. The reference collection contained 45% spawning individuals, but once transplanted the percentage of spawning individuals diminished after one month to 20% and after two months to merely 5%. No spatial pattern could be found.

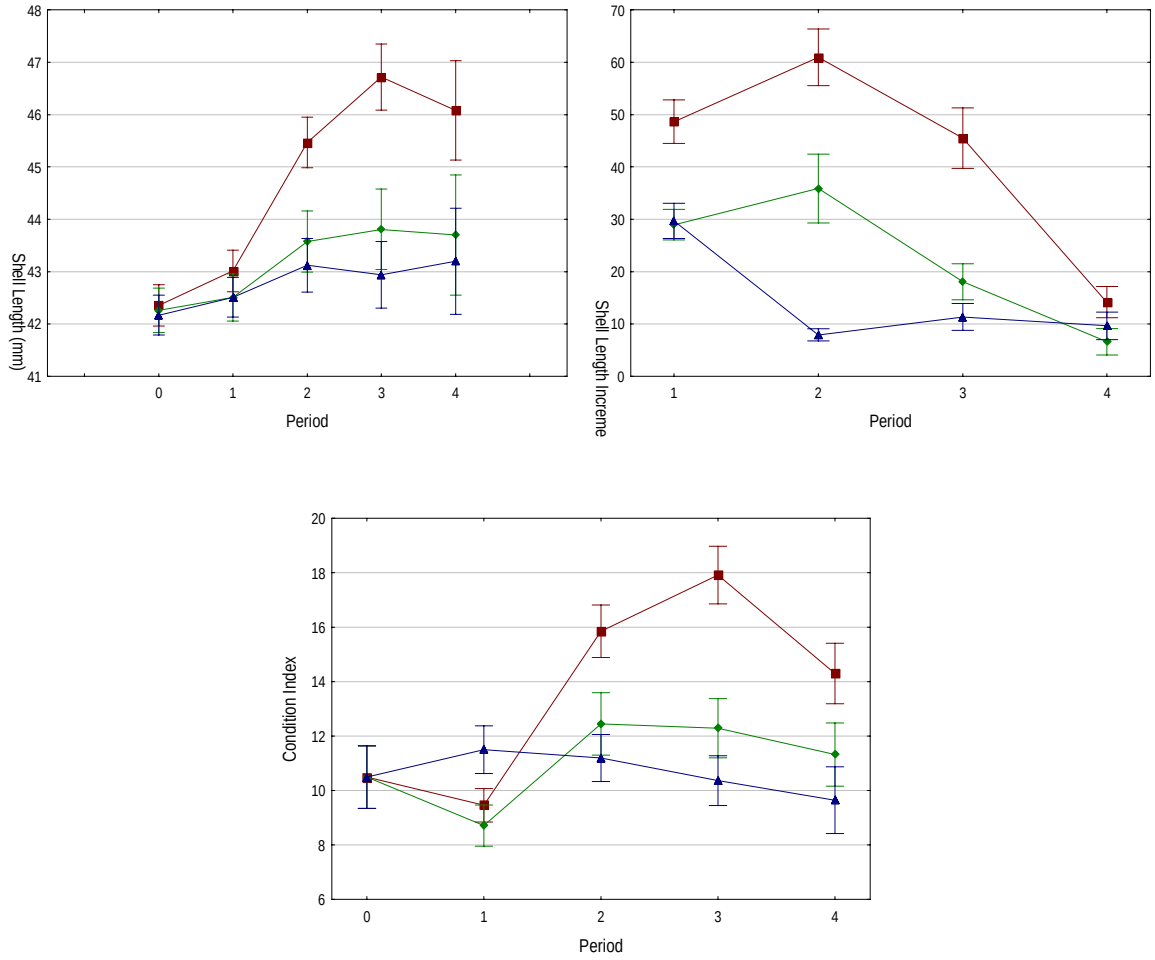

Figure 13. Shell Length, Shell Length Increment and Condition Index of *Mytilus edulis* Jun -Oct '07. Station ■ = ZB3, ▲ = ZB2, ◆ =OO1

5.3.2 Cage - Experiment Feb – Jul '08 (focus on *Mytilus edulis*)

The second cage experiment was conducted at the marina of Zeebrugge (ZB2), Oostende (OO2), Nieuwpoort (NP2') and a sea station in the mussel cultivating area (W03'). In general the results show a clear contrast between W03' and the marina stations in Shell Length Increment (SLI) and Condition Index 1 (CI1). With highest values in W03' after five months of exposure.

The biometric parameters (SL, SLI & CI) were statistically tested and they all showed a similar pattern. A one way ANOVA indicates no significant difference in the variables between the two replicas at each station ($p > 0.05$) but indicates a significant difference between the stations ($p < 0.05$).

With a non parametric Spearman Rank analysis, no significant correlation could be found between abiotic factors (temperature, salinity, oxygen concentration, chl a and spm) and the biometric parameters. Although it is remarkable that the SLI abruptly declines in OO2 after period 2, it also declines in ZB2 after period 3 and even stops in NP2'. This coincides with an oxygen depletion probably due to a heavy *Phaeocystis* spring bloom. When the oxygen drops below 4 $mg.L^{-1}$ negative effects on SLI and CI are observable.

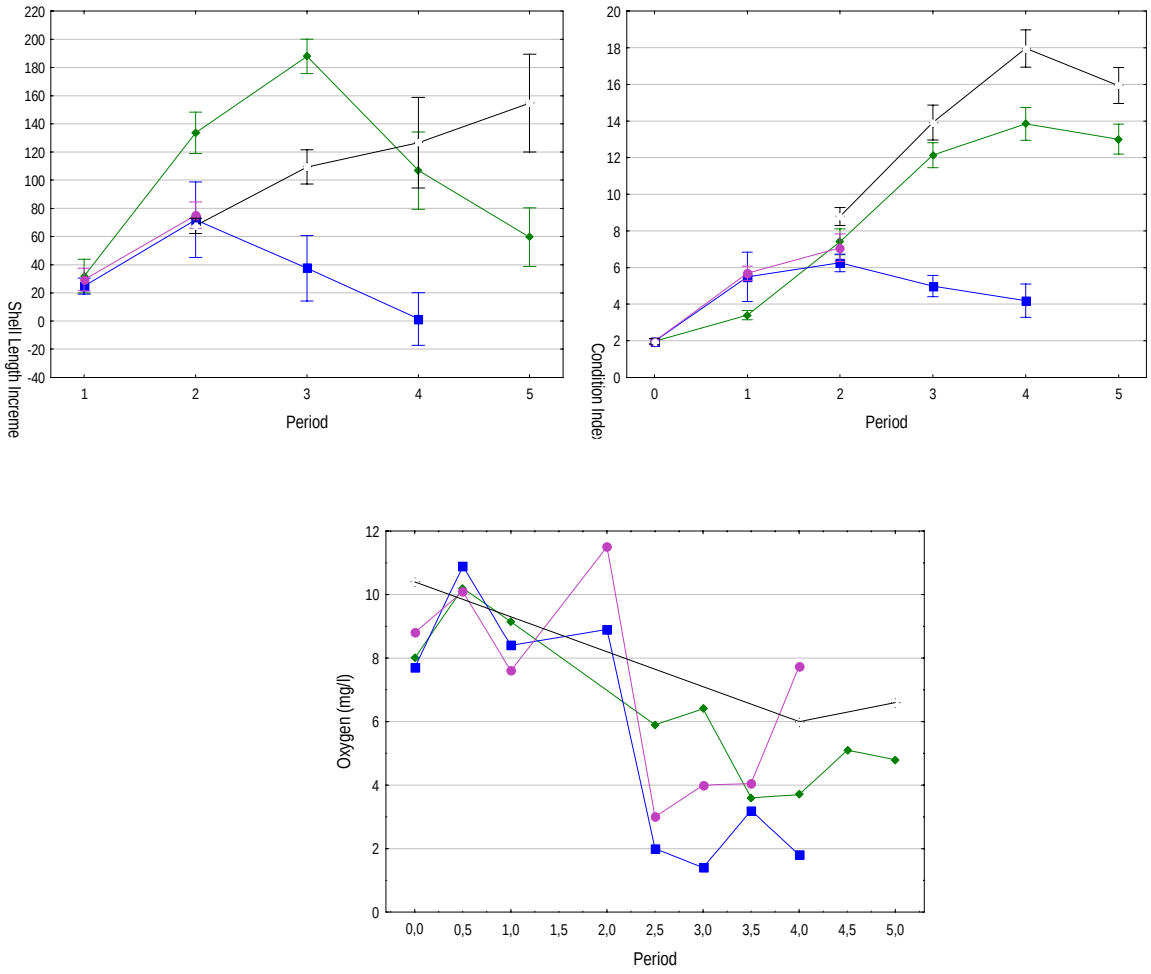

Figure 14. Shell Length Increment, Condition Index of *Mytilus edulis* and H_2O Oxygen concentration Febr-Jul '08 Station ┼= W03', ◆ = ZB2, ■ = OO2, ● = NP2'

Flatfish were sampled in order to collect livers for chemical analyses. *Limanda limanda,* larger than 18 cm, is the most appropriate species to be collected for the chemical analyses. During the winter campaign '07 and spring campaign of '08 an equal quantity of *Limanda limanda* was collected according to the fishing effort. Highest quantities were sampled at stations W03 and W05. The subsequent most abundant flatfish species was *Solea solea*, which is less appropriate for chemical analyses. High densities of *Pleuronectes platessa* were sampled during summer '07 at the stations W03 and S01, but almost no *P. platessa* of adequate size was sampled during the other campaigns. Depending on the results of the chemical analyses of the livers the question of the necessity of a higher fishing effort will be addressed for following sampling campaigns.

Table 3. Number of flatfish caught per fishing effort of sampling campaigns July '07, December '07 and April '07

JUL 2007	cm	W01	W02	W03	W04	W05	W06	S01
Solea solea	>20	x	9	4	0	1	0	22
Platichthys flesus	>15	x	2	0	0	0	0	0
Pleuronectes platessa	>20	x	0	12	0	5	3	19
Limanda limanda	>18	x	0	0	0	1	0	0

DEC 2007	cm	W01	W02	W03	W04	W05	W06	S01
Solea solea	>20	0	0	0	0	2	0	1
Platichthys flesus	>15	0	0	0	0	0	0	0
Pleuronectes platessa	>20	1	0	0	0	4	0	0
Limanda limanda	>18	0	2	6	0	6	0	0

DEC 2007	cm	W01	W02	W03	W04	W05	W06	S01
Solea solea	>20	6	6	0	0	1	0	4
Platichthys flesus	>15	0	0	0	0	0	0	1
Pleuronectes platessa	>20	0	0	2	0	0	1	0
Limanda limanda	>18	0	1	8	0	5	0	0

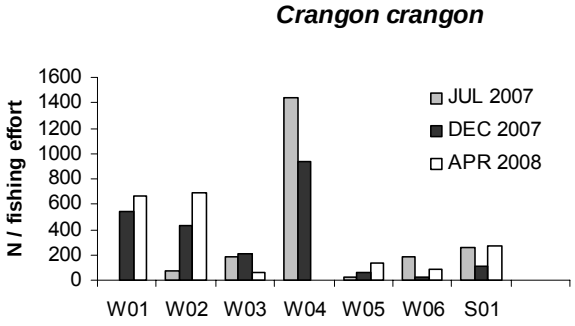

Figure 15. Number of *Crangon crangon* caught per fishing effort of sampling campaigns July '07, December '07 and April '07

Crangon crangon, was also sampled for chemical analyses. The highest abundance of brown shrimps was found at W04, i.e. the sea station in front of Zeebrugge, during the summer and winter campaign of 2007 with more than 1000 individuals per fishing effort. A relative higher abundance is found in the eastern coastal stations (W01, W02) and the stations located at the Scheldt estuary (S01, W04) in comparison with the western located stations further offshore (W05, W06).

The mysid species diversity in the hyperbenthos samples of the first two sampling campaigns (May-Jul '07, Nov-Dec '07) was as expected. *Mesopodopsis slabberi* and *Schistomysis kervillei* are abundant species, *Schistomysis spiritus* and *Gastrosaccus spinifer* are also present in the samples and even individuals of *Neomysis integer* and *Siriella armata* were observed. The density of the mysid shrimps, however, was very low. In the harbours the highest density reached was 31

individuals/m³ in Oostende for *Schistomysis kervillei* during the summer campaign '07. This shows that mysid shrimp densities are too low to be sampled adequately for chemical analyses.

Macrobenthos samples of the first sampling campaign (May-Jul '07) were sorted out, identified to lowest as possible taxonomic level and counted. In total 10254 individuals were encountered, representing at least 70 different species, belonging to 46 different families. A preliminary non-metric multidimensional scaling (MDS) analysis (Primer 5) is deployed to discern spatial benthic community patterns. Data were square-root transformed to reduce the skewed influence of species with very high abundance, prior to Bray-Curtis similarity index (Bray-Curtis, 1957). Performing the MDS on all stations, it becomes clear that euryhaline stations do separate from the other stations. As such we were obliged to eliminate the upmost brackish stations (S22, NP1 and NP2). The obtained MDS shows a slight distinction between sea stations (W03, W04 & W06) and coastal stations (S01, W01, W02 & W03). The outer harbour (ZB3, ZB4, OO4, NP3) lean towards the coastal stations. The remaining inner harbour stations are not clearly positioned, probably due to their brackish character. Further statistical analyses are necessary to reveal the obtained patterns.

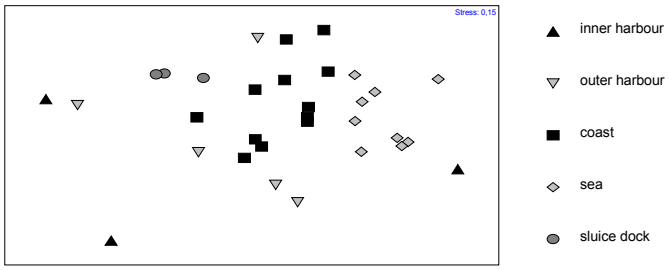

Figure 16. Preliminary MDS plot of Macrobenthic community May-July 2007

5.4 Risk assessment

For the 49 selected micropollutants, we have already collected physico-chemical, human, and ecotoxicological data. A summary can be consulted on the private part of the INRAM website: http://www.vliz.be/projects/Inram/private/documents/arcadisstoffen.pdf

Measured concentrations that were above the detection limit (dl = 0.1 μg.L^{-1}) of the applied methods are used as the PEC for risk characterisation (sampling May-June 2007). For atrazine and isoproturon, also data from sampling period December 2007 are used. PNECs are obtained by data gathering as described under methods.

The risk characterization ratio was calculated with the following formula:

$RCR_{freshwater} = PEC/PNEC_{freshwater}$

$RCR_{water,marine} = PEC/PNEC_{water,marine}$

Results are summarized in table 4. The PNECs for atrazine from different sources differ with a factor +/- 10. The lowest PNEC value has been selected (worst case scenario). The risk characterisation ratios (see last column) show that no risk for nonylphenol, atrazine, diuron and isoproturon are concerned at sampling period of May-June 2007/December 2007.

Table 4. Summary of risk characterisation of nonylphenol, atrazine, diuron and isoproturon of the aquatic compartment (May-June 2007)

Micropollutant	Sampling location	Measured concentration ($\mu g.L^{-1}$)	PNECaquatic ($\mu g.L^{-1}$)	RCR
Nonylphenol	S22	0,79	3,3 inland	0,24
	OO4	1,11	3,9 marine	0,28
	ZB4	0,78	3,9 marine	0,2
Atrazine	S22	0,024	0,95 inland	0,025
	S22	0,014*	0,95 inland	0,015
Diuron	S22	0,125	1,92 inland	0,065
Isoproturon	S22	0,033	5,2 inland	0,006
	S22	0,172*	5,2 inland	0,033

*: Data from sampling period December 2007

6 Discussion

6.1 Exposure assessment

6.1.1 Conventional chemical analysis

The research that has been performed in the first phase of INRAM has pointed out that multiple micropollutants occur in our marine environment at concentrations which often exceed environmental quality standards and potentially present a risk to Belgian marine systems.

6.1.1.1 PAHs

Concentrations of PAHs in sediments regularly exceeded the lower limit of the Ecological Assessment Criteria (EAC) for PAHs (stations OO1, OO2 and S22) although the upper limit of the EAC was never exceeded. The EAC was most frequently exceeded for pyrene. The compounds anthracene, fluoranthene and benzo(a)pyrene exceeded the EAC for PAHs in water in respectively 49%, 10% and 13% of the cases with a factor ranging from 1 to 16. The Annual Average Environmental Quality Standard (AA-EQS) (WFD directive 2000/60/EC), for water was exceeded for sum(BkF, Indeno) and sum(BbF, Bghi) in respectively 85% (factor 1-15) and 10% (factor 1-3) of the cases. At S22 and OO2 the EQS was exceeded with a factor of 30. The EQS for other PAHs was not exceeded.

6.1.1.2 Organotins

As the EAC (Ospar, 2000) for TBT in sediments is set on 0.05 μg.kg^{-1} dry weight, it exceeded in all stations with a factor 1 to 1600. The AA-EQS (0.2 ng.L^{-1}) and EAC (0.1 ng/L^{-1}) for TBT in water were exceeded in 79% of the cases. Also, the maximum allowable EQS (1.5 ng.L^{-1}) was exceeded in 47% of the cases.

6.1.1.3 BDEs

AA-EQS for penta-BDEs is 0.2 ng.L^{-1} and was exceeded in 83% of the cases in this study.

6.1.1.4 Phenols

The detected concentrations are in line with the typical estuarine and marine maximum concentrations of nonylphenol of 1 μg.L^{-1} reported in literature (Ferguson et al., 2001; Jonkers et al., 2003; Kvestak and Ahel, 1994). The EQS was exceeded at three sampling locations in May '07. In the harbours of Ostend and Zeebrugge and in the Scheldt in Antwerp, the presence of nonylphenol could be harmful for the marine environment.

6.1.1.5 Organonitrogen pesticides

For diuron the EQS was exceeded at sampling locations NP1 (harbour ofNieuwpoort), S22 (Scheldt estuary in Antwerp) and OO2 (harbour of Ostend). Additionally, the considered PNEC-

value of terbutylazine was exceeded in the Scheldt estuary near Antwerp (S22) and in the harbour of Ostend (OO2) in April 2008. There is a potential risk for the aquatic ecosystem where the detected levels exceed the EQSs (or PNEC-value).
Generally, the targeted ONPs were detected more frequently and in higher concentrations in the harbours and in the Scheldt estuary in Antwerp in comparison with the offshore sampling locations. In the harbours, there is a huge input of surface water containing certain levels of ONPs. ONP-concentrations lower towards the open sea. Additionally, more ONPs were detected in December. ONPs are used in spring and reach the surface water and consequently the marine environment by infiltration, spills, spray drift or run-off.

6.1.1.6 Pharmaceuticals

The obtained results showed that the Scheldt in Antwerp and the harbour stations (Zeebrugge, Nieuwpoort, Ostend) were most polluted with pharmaceuticals, irrespective of the sampling period. At sampling locations OO2 and S22, targeted pharmaceuticals were detected most frequently and in the highest concentration range. OO2 is situated in the harbour of Ostend on the mouth of the river Noord-Ede and the canal Brugge-Ostend. At this place huge amounts of surface water, possibly polluted with pharmaceuticals, reach the seawater in the Ostend harbour. S22 is situated in the Scheldt estuary at Antwerp, where the Scheldt receives major inputs of industrial and domestic waste water. Effluents of WWTPs are generally known as the main source of contamination of pharmaceutical compounds into the aquatic environment. In Flanders more then 65 WWTPs effluents reach direct or indirect the Scheldt. In de vicinity of the Scheldt estuary WWTPs with the largest capacity are located: Deurne, Ghent and Antwerp (approximately 200 000 IE = Inhabitant Equivalent, which is the daily amount of waste water produced by 1 inhabitant) (Aquafin NV, Nuytemans V., Personal Communication). The obtained results confirm our expectations, detecting highest concentrations in the vicinity of WWTPs.

Pharmaceuticals were detected up to 0.855 μg.L^{-1}. To the best of our knowledge, this is the first time pharmaceuticals were found in the marine environment in these concentration levels. The obtained results confirm the presence of pharmaceuticals in the North Sea and their ubiquitous character. This could lead to new insights into their persistence in the aquatic environment.

6.2 Effects assessment

6.2.1 Mixture toxicity testing with passive samplers

The results of the modeling experiments show that by adding 5g of PDMS passive sampler to 50 mL of test medium assures a depletion of no more than 10% for compounds with a log(Kd) value of 2 or higher in the sampler. For these compounds, it can be said that the concentrations in the medium reflect the environmental concentrations well, since the reversed equilibrium is not affected much by this depletion. Compounds with a log(Kd) value of two or more are the most important compounds since they are more hydrophobic and therefore accumulate more in organisms.
The release kinetics show that it takes no more than 5 hours for the compounds absorbed in the sampler to reach equilibrium with the uncontaminated test medium. With additional shaking, this time is expected to be even less.

Moreover, the mixture toxicity tests with contaminated passive samplers show a good reproducibility and similar results between test species. Severe adverse effects on the development of oyster larvae were observed for the mixtures originating from the harbours of Oostende (OO2 and OO3') and Zeebrugge (ZB2 and ZB3). Station OO2 was the only station for which 100% mortality of the oyster larvae was observed. The mixture from this station was the only one causing a significant inhibition of the growth rate of *P. tricornutum*. The growth inhibition test with *P. tricornutum* was repeated three times with contaminated passive samplers from the same sampling period and the same results were obtained each time. On average, station OO2 showed a growth inhibition of 12%. The results of both the modeling experiments and the ecotoxicity tests show that performing ecotoxicity tests in this way is both feasible and reproducible, allowing the test organisms to be exposed to environmentally relevant pollutant mixtures under controlled laboratory conditions.

6.2.2 *In situ* testing: *Mytilus edulis* biomarker analysis

The first results of the *in situ* biomarker experiments conducted with caged mussels in the three Belgian coastal harbours (Oct – Nov 2008), show different responses of the test organisms between the harbours. The CEA assay shows a reduced cellular energy allocation at the harbours of Oostende and Zeebrugge, which is in agreement with the generally higher pollution at these locations (than for example in Nieuwpoort). When looking in detail at the different energy reserves, more subtle differences can be discerned. The organisms deployed at Zeebrugge showed a reduction in lipid reserves; at the station in Oostende a reduction of carbohydrate levels was observed. A reduced lipid content has been linked to an increased exposure to PAHs (Yeats *et al.*, 2008), which could be an explanation for this observed effect. Also, an elevated EROD activity was observed at station ZB3, which is an indication of pollution by planar PAHs, PCBs, PBDRs and possibly others. Indeed, a higher pollution by PAHs was observed in Zeebrugge, both at station ZB2 and ZB3 (see Figure 7). At ZB3, the (freely dissolved) PAH contamination was only slightly higher than at OO2 (23 and 18 $ng.L^{-1}$ respectively), but at station ZB2, the sum of 22 PAHs reached a value of almost 120 $ng.L^{-1}$. This could confirm the earlier suspicion that the low EROD activity at station ZB2 is due to a high pollution by PAHS and the bell shaped Slightly higher values for brominated diphenyl ethers were observed at Zeebrugge, which could also be an explanation for this. Analysis of the passive samplers which were deployed simultaneously with the caged mussels, must bring more clarity. Furthermore, analysis of the remaining biomarkers (ALP, LMS and Metallothionein concentrations) could further unravel the causes of the observed adverse effects (e.g. on CEA or on ecological health endpoints).

6.2.3 Quantitative Toxicological Interpretation

Although the QTI that was performed in this first phase of the project was still very rudimentary – i.e. based on literature ecotoxicity data and using results of chemical analysis of water samples instead of passive sampler extracts – the results are very promising. The sums of TUs of the contaminant mixtures originating from the different sampling stations, reflect the toxicity gradients observed in the assays conducted with contaminated passive samplers closely. Some inconsistencies remain, e.g. even though the toxic effect on the development of oyster larvae was much higher at ZB2 than at both other stations, the sum of TUs at station ZB2, was lower

than at both NP1 and NP2' for the 24h larval development test with *C. gigas*. The use of other mixture toxicity models will be examined in the second phase of the project, as only concentration addition was used. This could partly explain the current inconsistencies. Also, not all groups of micropollutants have been taken into account yet. For example, pharmaceutical compounds were not yet included in the QTI analysis. It is expected that, once these and possible other missing compounds are included, the QTI analysis will improve.
However, it can also be expected that, with the final QTI method, strong synergistic or antagonistic effects can cause results differing from the ecotoxicological test results. The same can be true when unknown but very toxic compounds are present in the environmental pollutant mixture. This is, however, not a shortcoming of this technique but a benefit. In the first place, QTI can be used to unravel the toxic composition of a complex mixture of contaminants, offering information on which toxicants are the greatest contributors to the overall toxicity. In this way, local occurrence of contaminants is coupled directly to adverse effects on the health of (resident) marine species. Secondly, any inability to fully explain the toxicity of such a mixture with QTI, points towards the presence of an unknown component or interaction between components in the mixture. This way, even though it cannot identify them, QTI allows for the detection of unknown threats present in the environment. This makes it a very useful tool for monitoring applications.

6.3 Ecological monitoring

A first cage experiment with the mussels and oysters revealed clear differences in overall fitness of marine bivalves within a gradient from outer harbour to inner harbour. A second experiment showed a clear difference between the bivalves exposed at a sea station in comparison with the bivalves exposed at the marinas of the different harbours. Moreover, the results of the cage experiments show more severe effects on the overall fitness in the more polluted areas (e.g. the Sluice Dock of Oostende and the Yacht Harbours/Marinas).

The benthos monitoring results, at present, in a good supply of brown shrimp and livers of flatfish for the chemical analyses. Depending on the results of the chemical analyses the question of the necessity of a higher fishing effort will be addressed. On the other hand, the densities of mysid shrimps caught during the integrated sampling campaigns are too low to be sampled adequately for chemical analyses.

Preliminary results of the analyses of the macrobenthos diversity show a difference in species diversity of the brackish stations in the inner harbours in comparison with the outer harbour stations which, on their turn, are slightly different from the sea stations.

6.4 Preliminary risk assessment

The preliminary risk characterization performed according to the REACH Implementation Project (RIP) 3.2.2 Part E: Risk Characterisation didn't reveal any risk at the respective sampling locations for nonylphenol, atrazine, diuron and isoproturon for the sampling periods of May and June 2007 and December 2007.

7 Data management and outreach

As VLIZ was data-manager of the project ENDIS-RISKS, VLIZ was also addressed concerning the management of all informative and monitoring data and the communicative aspects within the INRAM project.

One of the main management objectives of INRAM was to increase the data availability and possibilities for exchange of data, but more importantly the setup of an integrated **ENDIS-RISKS/INRAM database**. This was realized through the development of a project website (with restricted and public pages), two separate project meta-databases, an integrated database and a Marine Data Archive (MDA). The project website (*http://www.vliz.be/projects/inram/*) is hosted and maintained by VLIZ. The website holds information on the **project planning**, **background, meta-information** and gives access to the **database**. Furthermore, some useful information, such as an overview of all cruises and reports and a detailed literature list of co-authored papers resulting from the project, can be downloaded from the website. The website has also become an important tool to access the **MDA** (see Figure 17). This backup and storage system is only available for project partners and gives the opportunity to archive all files created during the project.

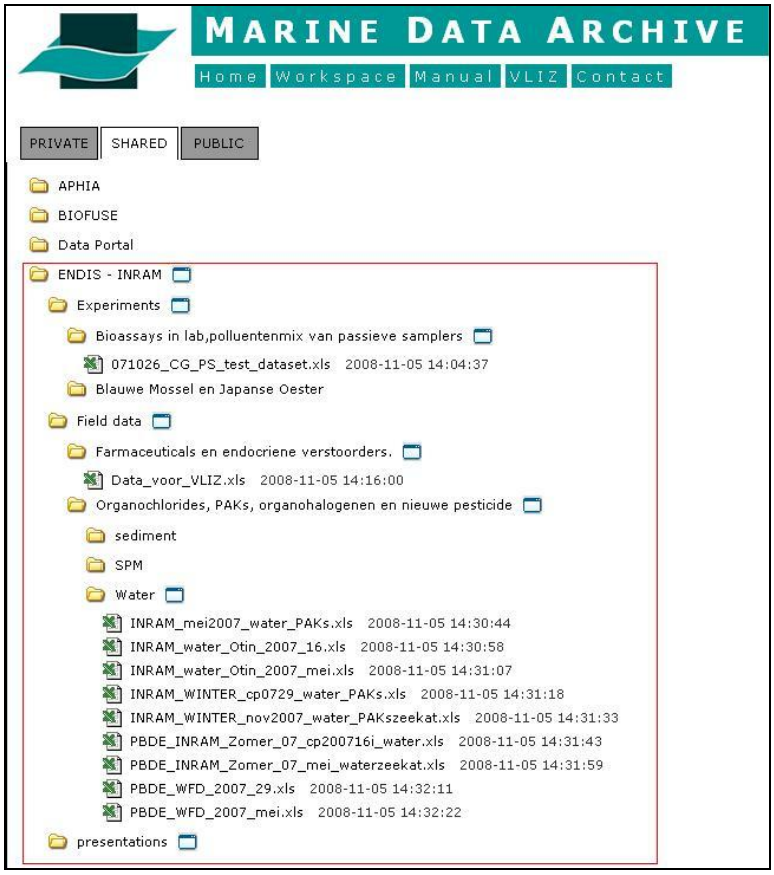

Figure 17. Screenshot of the Marine Data Archive.

The purpose of the Marine Data Archive is to provide a back-up and storage system for files related to marine sciences, whether it are documents, programs, graphs, presentations, ... Only persons who have access to the INRAM restricted area can consult or upload the files that are

stored in the Shared folder INRAM.
A structure with subfolders was made, matching the datasets within the project, to create a visually attractive archive. Each file has metadata information provided by the file author (see Figure 18). In this way it is very easy to browse through the folder and find the file needed.

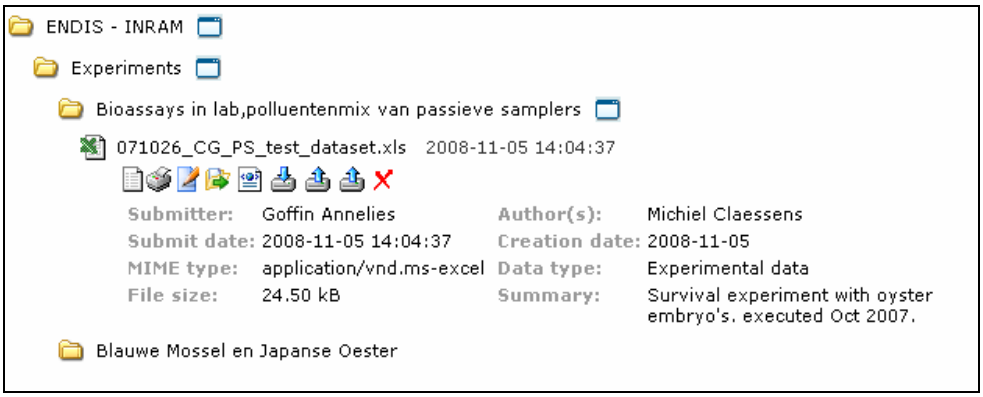

Figure 18. Each file added to the Marine Data Archive, has metadata information provided by the file author

The INRAM metadata-database contains an inventory of 11 datasets, generated within the project. Furthermore it also contains information on persons, institutes, publications and conferences relevant to the project. The set-up of the database as well as the input of the content was done in collaboration with the project partners.
(http://www.vliz.be/projects/inram/imis.php?module=dataset&show=search)

Datasets within the INRAM project:
- INRAM laboratory bioassays: single pollutants and prepared pollutant mixtures
- INRAM laboratory bioassays: pollutant mixture from passive samplers
- INRAM field bioassays
- INRAM Blue Mussel and Giant Pacific Oyster studies
- INRAM concentrations of pharmaceuticals, diruon, isoproturon and endocrine disruptors in the Scheldt estuary, Belgian coastal harbours and Belgian coastal waters
- INRAM concentrations of organochlorines, PAHs, organohalogens and "new" pesticides in the Scheldt estuary, Belgian coastal harbours and Belgian coastal waters
- INRAM concentrations of metals in the Scheldt estuary, Belgian coastal harbours and Belgian coastal waters,
- INRAM benthic fauna monitoring
- INRAM pollutant concentrations in tern eggs, sampled in Zeebrugge
- INRAM project data (2007-2010)
- INRAM mysid population studies
- Supporting environmental data of the INRAM sampling campaigns.

The integrated ENDIS-RISKS/INRAM database holds the actual field data that was gathered during the project. The set-up of the database is suitable for storing both physical and non-physical data. Experimental data is not integrated into the database, but made available for projects partners on the MDA. The measured values or readings are integrated in such a way

that data on the corresponding trip or cruise, visit to station, sample, methodology, data-originators, can easily be consulted. All data is also linked to standard vocabularies like the European Register of Marine Species (ERMS) taxonomic reference list and the European marine gazetteer. This increases the possibilities for (international) exchange of data.

Most of the ENDIS-RISKS data is made available through the public website by means of a user-friendly tool *(http://www.vliz.be/projects/inram/imers.php)* as also described in the ENDIS-RISKS report. INRAM data is only available on the restricted pages of the website. The data policy is the same as in the ENDIS-RISKS project. Data can only be made available two years after sampling event. The policy document was drawn up based on input of all project partners.

By downloading or consulting data from the website, the visitor acknowledges that he/she agrees with the data policy as it is stated here:

If data are extracted from the INRAM database for secondary analysis resulting in a publication, the INRAM database should be cited. The INRAM database should be cited as follows:

Laboratory of Environmental Toxicology and Aquatic Ecology, Ugent; Management Unit of the North Sea Mathematical Models; Laboratory of Chemical Analysis, Ugent; Marine Biology Section, Ugent. Belgium. *INRAM. Integrated Risk Assessment and Monitoring of micropollutants in the Belgian coastal zone - SSD - Belgian Science Policy. Available online in IMERS at http://www.vliz.be/projects/inram/imers.php. Consulted on 15-01-2009.*

If more than 10% of the data are derived from an individual datasource that is part of the parent database, the data can be used but the individual datasource should be cited as well. Dataset Citations are included as a record in the exportfiles.

If more than 25% of the data are derived from an individual datasource that is part of the parent database or the data are essential in the analysis, individual datasources should be cited. Before using the data the manager/custodian of this dataset should be contacted.

Citation references are given on the meta-dataset information sheets.

Meanwhile, the ENDIS-RISKS/INRAM database contains a substantial amount of data. All together the database stores **75301** measured values or so called 'readings'. The experimental data is not included. **69867** readings were measured during ENDIS-RISKS, **5434** readings during INRAM. Because INRAM has a lot of experimental work many values will not we integrated into the database. Nevertheless lot's of readings are expected from INRAM field work at the end of 2010. **66219** of these readings are linked to specimens or to biotic records. The ENDIS-RISKS/INRAM database stores **13066** chemical readings: **2168/3499** in the water column, **1935/1411** in the sediment and **3569/524** in suspended matter.

The data sampled during the INRAM project are gathered from project partners and integrated in the IMERS database of VLIZ as an INRAM collection, before being reported to the IDOD database of BMDC. In preparation of the integration, data is completed, if necessary, with missing metadata information (data concerning campaigns and stations, sampling and analysis methodologies). The integration of the data is preceded by quality control. Missing, duplicate and suspect data and outliers are identified and adjusted in agreement with the data author. Changes or suspect data are never deleted. Quality flags act as criteria when querying the data. Because VLIZ was involved in the ENDIS-RISKS data management, no further structural adaptations of the IMERS database were needed to integrate the INRAM data.

Throughout the project, the entire consortium needs to be involved within the data management. It remains important to have close collaboration between scientists and data managers throughout the duration of the project.

Outreach

The creation of a project website and the visibility of data are part of the outreach of the INRAM project. VLIZ uses its communication channels to the broader public to give special attention to the science used within the INRAM project.

Together with VLIZ the Dutch foundation ZeeInZicht is working on an Educational website on Marine ecology (*http://www.zeeinzicht.nl*). This portal to the VLEET (Marine encyclopedia from Ecomare) was updated and different INRAM outreach products were made and will be linked with this website to promote the research within INRAM.

Ecotoxical information will be incorporated into the Coastal Wiki of Encora. In close cooperation with Ecotox scientists, the content will be outlined. Structural and technical aspects and the promotion of the part of the Wiki will be done by VLIZ.

8 Conclusions and second phase perspectives

The INRAM project aims to develop a new method to perform an integrated risk assessment of micropollutants in the Belgian coastal zone. To this end, different research disciplines are being integrated in a unique way. This first phase of the project consisted for the most part of data collection. The gathered data will be fully integrated in the second phase of the project. However, the sampling campaigns, chemical analysis and laboratory and field experiments have been designed and organised in such a way to allow an easy integration of the resulting data. This is already apparent from the results of the first phase, which show good agreement between local pollution observed through the sampling campaigns and subsequent chemical analysis, and the local health status of the marine ecosystems observed through ecological and ecotoxicological laboratory and field experiments. Perhaps one of the most crucial observations is that, even though preliminary, the performed risk characterisation did not reveal any chemicals that are posing a risk to the marine environment according to the current evaluation methods. Yet, the ecological and ecotoxicological studies conducted in this first phase of the project, do show clear pollution-related adverse effects. This confirms that complex mixtures of pollutants can cause adverse effects on organisms, even when the individual components of the mixture are present at concentrations well below their effect levels.

One of the most important tasks in the second phase, is the completion of the chemical analysis of passive samplers and biota in order to allow a complete integration with the available effect data.
Based on the already available data on the occurrence and distribution of pollutants, additional effect data will be generated for individual priority compounds. This will allow for a more realistic QTI analysis to be performed, resulting in an enhanced capability to identify the components in the complex environmental mixtures which are responsible for the observed adverse effects on the test organisms. Since mixtures of this kind were collected simultaneously with the *in situ* biomarker experiments with caged mussels, we will be able to compare these results to (1) data on concentrations of pollutants in mussel tissue, (2) the results of the biomarker analyses, providing effect data related to different kinds of chemical classes, and (3) the ecological health endpoints and draw possible parallels.
For the ecological studies, benthos samples will be further worked out and analyzed in order to allow a spatial and temporal comparison in diversity of macrobenthos, epibenthos and mysid shrimps. In addition, benthos monitoring results will be coupled with environmental and contamination data. More focused bivalve cage experiments will be performed based on the findings of the chemical analyses of the biota, the water samples and the passive samplers originating from the first two cage experiments. The relationships between the overall fitness of the bivalves and the contamination data will be further analyzed.

9 Acknowledgements

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