SSD

SCIENCE FOR A SUSTAINABLE DEVELOPMENT

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

«INRAM»

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North Sea

FINAL REPORT

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

SD/NS/02

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SUMMARY

Context

Despite extensive research efforts, little is known about the transfer of chemical pollutants 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 sometimes 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.

Objectives

The overall aims of the INRAM project were to (1) study the environmental concentrations of established priority compounds (cf. OSPAR, Water Framework Directive (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.
Central to several of the innovations envisaged to result from this project, were passive samplers. Passive samplers made of polydimethylsiloxane (PDMS) were 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 (additionally, its potential to function as a surrogate for sediment and SPM was also explored); and (c) as a contaminant source for dedicated ecotoxicological (laboratory) assays allowing exposure of organisms to environmentally relevant chemical mixtures.

Conclusions

All main goals of the project were achieved and the project can thus be considered a major contribution in the assessment of the environmental quality of Belgian coastal waters and in the development of new, innovative techniques and approaches to assess, monitor and manage marine ecosystems. The presence and concentration of more than 100 chemical – existing and emerging - substances in various matrices (including biota) of different parts of the Belgian coastal system (harbours, coastal waters and Scheldt estuary) was quantified. The possible effects of these substances (single and mixtures) was assessed using a combination of ecotoxicological assays, biomarkers, in situ experiments and biodiversity surveys. The multitude of data was integrated in the environmental approach which – for the first time ever – allowed to assess the risks posed by these substances to ecosystem health and human health. In can be concluded that as much a 25% of the micropollutants analyzed present in our coastal waters to pose a risk to organisms living in the water and sediment compartment. As expected harbours are more contaminated and thus present a higher risk to ecosystem health. The project also – for the first time ever - demonstrated that the novel approach (developed in this project) with passive samplers can be used to assessment environmental and human health risks. As such the project now proposes a new and simple approach to evaluate the health status of coastal ecosystems.
The main achievements can be summarized as follows:

- Novel analytical methods were developed and existing methods were improved during the course of this study.

- These have allowed obtaining an assess the presence and distribution of a suite of hazardous substances, some previously undetected, in the major ports and along the Belgian coast.
- Passive sampling has proven to be a valid alternative to classical monitoring and a promising dosing tool for effects monitoring.
- Effects monitoring (in situ and in vivo) shows distinct differences between the harbours, validating it's use in an integrated monitoring scheme.
- There is a clear difference in the abundance and distribution of species between the investigated area's. This could partly be related to pollution illustrating the need to include ecological monitoring in an integrated scheme.
- Clear progress was made in the risk assessment of the substances under investigation.
- Including consumer species and top predators in the sampling, allowed assessing the risks for the highest trophic levels, including man.
- Distribution of contaminant between the compartments through the dissolved phase (simulated by passive samplers) has proven to be a valid working hypothesis, predicting the presence and distribution of hazardous substances in the marine environment.
- The project has laid the foundations for integrated monitoring and risk assessment in the Belgian coastal area.

Contribution of the project in a context of scientific support to a sustainable development policy

Belgium has the obligation to assess the status of its coastal area in compliance with the EU Water Framework Directive (WFD) and the International Convention on the Protection on the North East Atlantic or the OSPAR convention. Furthermore, the recent Marine Strategy Framework direction (MSFD) will require Belgium to assess whether its coastal and marine waters have a good environmental status (GES). Both the WFD and MSFD further require members states make provisions to achieve a good environmental status by 2020 if this is not already the case. Central for these assessments, is the availability of quality data provided from monitoring programmes. Monitoring is one of the most important management tools used for assessing the quality status of an area and changes thereof (i.e. temporal trends). Over the past 40 years the objectives of monitoring have changed into an ecosystem approach where – in Europe under the WFD and MSFD – Good Environmental Status shall be reached. This Ecosystem Approach led to a different type of assessment. The objective now was to assess the pressures of human activities as well as the resulting impacts on, and state of, the marine

environment. For hazardous substances, the goal is to obtain a comprehensive risk assessment for man and the environment.
In response to this more holistic demand and from a purely scientific perspective, there is general agreement that the best way to assess the quality status of the marine environment, with respect to hazardous substances, is by using a suite of chemical and biological measurements in an integrated fashion. In this respect, biological effects techniques have become increasingly important in recent years. Furthermore, passive samplers have gained prominence among the novel techniques that attempt to assess the status of a given environment with regard to hazardous substances. Furthermore, not all adverse effects will be observed solely by looking at concentrations of contaminants or their known effects. One needs to establish a good knowledge about the presence and distribution of species in any given enviroment hence the need for biological monitoring.
This project aims to take the next step in developing true integrated monitoring in Belgian coastal waters by looking at the different levels mentioned above and, by using both conventional and novel approaches. As such, it supports Belgian policy makers in their obligations of assessing the quality status of our waters as well as boosting research in this field. The outcome is also highly relevant for progressing integrated monitoring at the international level i.e. OSPAR and MSFD level.

Keywords

Integrated risk assessment; Chemical monitoring; Ecological monitoring; Marine environment; Micropollutants; Passive sampling; Passive dosing; Biomarkers; Water Framework Directive; OSPAR; Sediment; SPM; Marine organisms

1. 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 that affect the hormone systems of animals and their offspring, the so-called endocrine disruptors, have attracted the worldwide 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 polychlorinated biphenyls (PCBs), dioxins and organotins, are 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).
Some of the main reasons for this are: (1) monitoring of these chemicals is sometimes 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 are 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, Water Framework Directive (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 INRAM project can be divided into four main parts:

1.1. Exposure assessment – sampling and chemical analysis

Sampling stations selected in the three Belgian coastal harbours, on the Belgian Continental Shelf (BCS) and in the Scheldt river are sampled at least once per year during the course of the project. The sampling stations are chosen in order to enable the study of pollutant transfer from the harbours and the Scheldt river to the North sea. On each sampling station, water, sediment, biota and suspended particulate matter (SPM) are sampled.

All samples collected during the sampling campaigns are chemically analyzed using chemical analytical procedures previously established in the partners' laboratories (cf.

ENDIS-RISKS network). Some of these techniques are further refined (e.g. clean-up of extracts from biological tissues) and new techniques are developed for the analysis of emerging pollutants (e.g. pharmaceuticals). 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 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 (PDMS) 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 (additionally, its potential to function as a surrogate for sediment and SPM will also be explored); 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 is 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 based on the review by Roose and Brinkman (2005). These authors based their recommendations and selection mainly on the EU WFD list. However, in contrast to the conclusions of the WFD, some additional OSPAR priority substances and the 'old' organochlorines are still included, partly because they are on the UNEP-POP list but also because of their environmental relevance. Tributyltin (TBT) and some selected pharmaceuticals are 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.

1.2. Effect and bioaccumulation assessment

To assess the effects of the contaminants present in the Belgian marine environment, a set of organisms is exposed to either individual 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*, are studied. A novel exposure technique using PDMS sheets as a passive dosing device, allows exposure of the organisms to mixtures closely resembling true environmental pollutant mixtures. These contaminated devices are 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 bioassay 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 (*M. edulis*) are used to assess the effects of contaminants in field situations. A battery of five biomarkers is used to allow interpretation of the observed effects based on the occurrence of contaminants in the local environment. Simultaneously, PDMS sheets are 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.

1.3. 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 (*M. edulis*) and pacific oysters (*C. gigas*) are conducted to study biometric parameters (growth, condition indices and gonadal development) of the organisms in relation to contaminants present in the field. In a first experiment, a gradient from an inner harbour to an outer harbour is studied. A second more elaborate experiment elucidates the difference between harbour stations and an open sea station.

Ten eggs of the common tern (*Sterna hirundo*) are collected every spring from a breeding colony in the harbour of Zeebrugge. The eggs are analyzed for micropollutants.

Beside the study of the focus organisms, epibenthic, macrobenthic and hyperbenthic fauna is sampled. As such, a gradient in density and diversity for the different ecological compartments of the benthos is assessed, in relation with a possible contaminant gradient, from the inner harbours to the open sea.

1.4. Integrated risk assessment

The results of the different tasks are incorporated into an integrated risk assessment for Belgian coastal waters (including harbours) of the selected contaminants. Using the results of the extensive chemical monitoring of 'conventional' and 'new emerging' micropollutant (task 1) combined with the novel laboratory and field effect assessments (task 2) and the ecological assessment endpoints (task 4) the impact of the presence of these contaminants on Belgian coastal on the health of marine ecosystems, important coastal bird populations and man (through consumption of marine products) are assessed in a quantitative manner. The potential risks of mixtures of low level contamination by persistent micropollutants to marine ecosystems and man are also assessed.

Additionally, this research provides information to assess the relevance and validity of the currently used procedures to derive Predicted No Effect Concentrations (PNEC) (EU, Existing Substance Directive and REACH) and/or Ecological Assessment Criteria (EAC) (OSPAR) and/or Environmental Quality standards (EQS) established in the context of the EU WFD.

2. METHODOLOGY

2.1. Exposure assessment

2.1.1. Sampling

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 Figure 1 and Figure 2), were sampled three times in 2007 and once in 2008, 2009 and 2010, respectively.

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 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 offshore (at least 5 kilometres from the coast) to assess the contaminant gradient.

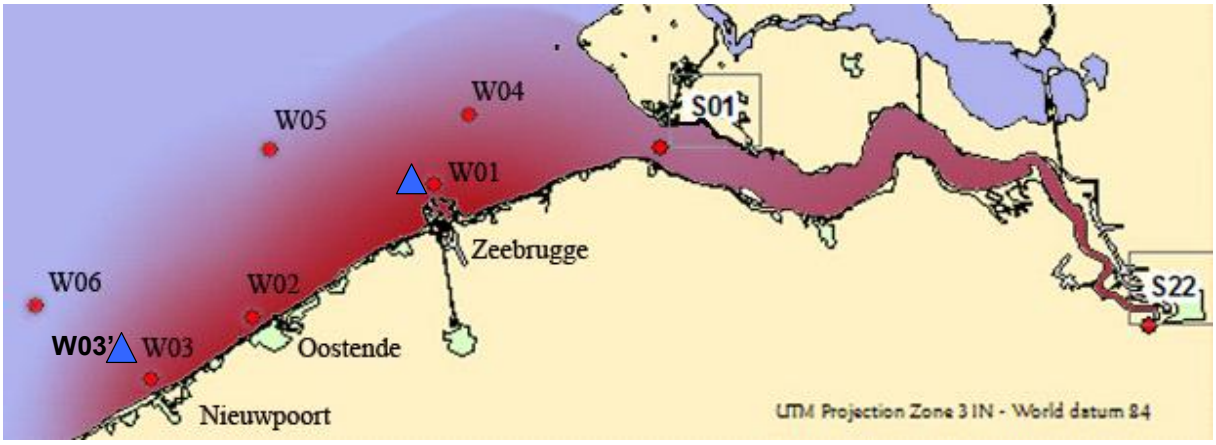

Figure 1. Sampling stations in the North Sea and the Scheldt river. ●: sampling station. ▲: cage with passive samplers and/or bivalves deployed.

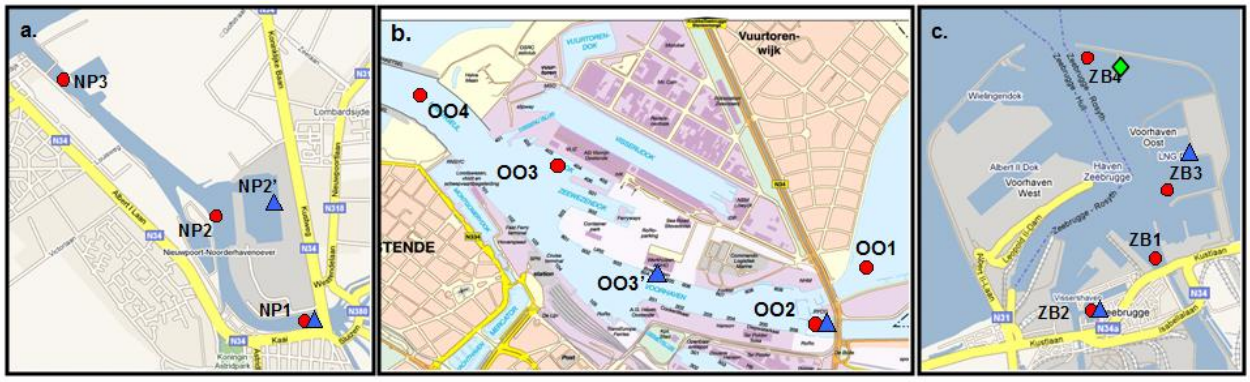

Figure 2. Sampling stations in the harbour of (a) Nieuwpoort, (b) Oostende and (c) Zeebrugge. ●: sampling station. ▲: cage with passive samplers and/or bivalves deployed. ◆: tern peninsula.

On each sampling station, water, sediment, biota and suspended particulate matter (SPM) were sampled. Several trophic levels (shrimp, mussel, oyster, fish and tern eggs) were considered as 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 and 2), either the research vessel (RV) 'Belgica' or the RV 'Zeeleeuw' was used.

Upon arrival at the sampling location, a CTD (conductivity – 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.
Finally, tern eggs were collected in the breeding colony of the Common tern in the harbour of Zeebrugge located on an artificial peninsula, called the Tern-peninsula. The peninsula is situated not far from sampling station ZB04, within the protection of the eastern dam and alongside the sailing channel.

2.1.2. Chemical analysis: newly developed techniques

2.1.2.1 Analysis of pharmaceuticals in seawater samples

Ketoprofen (99.0%), mefenamic acid (>99.0%), carbamazepine (>99.0%), diclofenac (>99.0%), bezafibrate (≥98.0%), salicylic acid (>99.0%), clofibric acid (97.0%), atenolol (≥98.0%), trimethoprim (≥98.0%), chloramphenicol (≥99.0%), and sulfamethoxazole (99.0%) were purchased from Sigma-Aldrich (St-Louis, MO, USA). Ofloxacin (>99.0%) was obtained from ICN Biomedicals Inc. (Ohio, USA) while propranolol (>99.0%) was purchased from Eurogenerics (Brussel, Belgium). The synthetic isobutcar 61 (4-3(isobutylamino-2-hydroxy-propoxy)carbazole) was found to be a very suitable internal standard for this application as it corrected sufficiently for any matrix effects in sample preparation and mass spectrometry.

Acetone, methanol and formic acid were of analytical grade quality and purchased from VWR (Merck, Darmstadt, Germany). HPLC grade acetonitrile and water were obtained from VWR (Merck, Darmstadt, Germany) and Acros Organics (Fairlawn, New Jersey, USA) respectively. Primary stock solutions of all individual analytes were prepared in ethanol at a concentration of 1 ng μL^{-1}. Working standard mixture solutions were prepared by appropriate dilution of the stock solutions in ethanol. All solutions were stored at -20 °C in the dark.

The developed extraction technique is based on 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 (1 M) and NaOH (1 M). Seawater samples of 1 L were filtered through a Whatman filter paper (GF/C Ø 47 mm, particle retention 1.2 μm, Merck, Darmstadt, Germany) to avoid clogging of the sorbent. The used filters were washed with 2 mL methanol to prevent loss of the compounds of interest. This methanol extract was collected and added to the filtered sample. Prior to extraction, the internal standard isobutcar 61 was added to every sample at a final concentration of 50 ng L^{-1}. Subsequently, 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 pre-conditioned with 5 mL methanol and 5 mL MilliQ water. After loading with 1 L of the filtered sample pooled with the methanol from the filter washing, the cartridges were rinsed twice with 5 mL MilliQ water. Subsequently, the cartridges were dried under vacuum for 30 minutes. Elution was performed using 5 mL acetone and 2 x 5 mL methanol. Next, extracts were dried using nitrogen and residues were reconstituted in 300 μL acetonitrile/0.02M formic acid (50/50) before transfer to LC-MS vials.

Analysis was carried out using high performance liquid chromatography (HPLC). The apparatus comprised of 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 (5 μm particle size, 250 × 4.0 mm; Macherey-Nagel GmbH & Co. KG, Düren, Germany). The void volume of the system was 2.5 mL. A filter (porosity 2 μm, 4 mm; Alltech, Waukegan, IL, USA) and pre-column (Nucleodur® C18, Isis, Macherey-Nagel GmbH & Co. KG, Düren, Germany) were used to prevent rapid deterioration of the analytical column. 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 linearly from 40% to 100% B in 20 min. 20μL was injected onto the LC-MSn system. Analytes were detected with a LCQ DECA Ion Trap mass spectrometer equipped with an electrospray ionisation (ESI) interface (ThermoFinnigan, San José, CA, USA) using the optimised MS-parameters described in the results section. Optimal ionisation source working parameters were: sheath gas flow rate, 80 arbitrary units (a.u.); auxiliary gas, 20 a.u.; capillary temperature 350 °C; capillary voltage, -14 V; and tube lens offset, 20 V. Chromatograms and spectra were recorded and processed using Xcalibur® 2.0 software (Thermofinnigan, San José, CA, USA).

Table I. Validation results: limits of quantification (LOQ), correlation coefficients (R^2), recovery and precision in seawater and tap water of the targeted pharmaceuticals.

Compound	LOQ ($ng\ L^{-1}$)	R^2	Seawater (n = 42)		Tap water (n = 6)	
			Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Salicylic acid	5	≥ 0.99	108	27	98	14
Mefenamic acid	5	≥ 0.99	95	20	92	14
Ketoprofen	50	≥ 0.99	103	18	109	9
Diclofenac	50	≥ 0.99	101	17	104	14
Clofibric acid	5	≥ 0.99	97	18	94	10
Bezafibrate	5	≥ 0.99	98	27	93	11
Sulfamethoxazole	10	≥ 0.99	100	19	106	17
Trimethoprim	10	≥ 0.99	108	19	96	12
Chloramphenicol	5	≥ 0.99	101	17	102	10
Ofloxacin	50	≥ 0.99	97	18	92	14
Carbamazepine	5	≥ 0.99	101	19	100	5
Propranolol	1	≥ 0.99	107	18	94	7
Atenolol	50	≥ 0.99	104	16	105	14

The newly optimized method was developed according to guidelines Commission Decision 2002/657/EC (2002) and SANCO/825/00 rev.7 (2004). The limits of quantification (LOQ) varied between 1 and 50 ng L^{-1}, the linearities were for most components more than 0.99 and recoveries obtained in seawater (95–108%) were satisfactory. The relative standard deviations (% RSD) were for most compounds satisfying as well (% RSD ≤ 20). The RSDs of bezafibrate and salicylic acid were somewhat higher at the lowest concentration levels. This can be attributed to the unavailability of a representative blank sample and consequently to the variation of its presence in unspiked water samples (Table I).

2.1.2.2. Analysis of perfluorinated compounds in seawater samples

Fourteen perfluorinated compounds (PFCs) were examined in this study: four perfluorosulfonates (potassium perfluoro-1-butane sulfonate, sodium perfluoro-1-hexane sulfonate, sodium perfluoro-1-octane sulfonate, and sodium perfluoro-1-decane sulfonate), nine perfluorocarboxylates (perfluoro-n-pentanoic acid, perfluoro-n-hexanoic acid, perfluoro-n-heptanoic acid, perfluoro-n-octanoic acid, perfluoro-n-nonanoic acid, perfluoro-n-decanoic acid, perfluoro-n-undecanoic acid, perfluoro-n-dodecanoic acid, and perfluoro-n-tetradecanoic acid) and perfluoro-1-octane sulfonamide. All analytical standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada) with chemical purities of more than 98 %. Six 13C-labelled internal standards were used as well: sodium perfluoro-1-[1,2,3,4-13C4] octane sulfonate, perfluoro-n-[1,2-13C2] hexanoic acid, perfluoro-n-[1,2,3,4-13C4] octanoic acid, perfluoro-n-[1,2,3,4,5-13C5] nonanoic acid, perfluoro-n-[1,2-13C2] decanoic acid, and perfluoro-n-[1,2-13C2] dodecanoic acid.

Methanol was purchased from Rathburn Chemicals (LTd Walkerburn, Scotland), while HPLC-grade water was obtained from Biosolve (Biosolve Chemicals, The Netherlands). Ammonium acetate (2.5 mM) in water was obtained from dilution of LC-MS Chromasolv® water containing 0.1 % ammonium acetate (Sigma-Aldrich Laborchemikalien GmbH, Seelze). Except for PFOSA, primary stock solutions of all individual analytes were prepared in methanol at a concentration of 50 μg mL^{-1}. PFOSA was purchased in nonane at the same concentration. Working standard mixture solutions were prepared by appropriate dilution of the stock solutions in methanol. All solutions were stored at -20°C in the dark.

The sample preparation protocol was based on the ISO 25101/2006 method (2006), which was in its turn derived from the method of Taniyasu et al. (2005). Sewage water samples and water samples, visibly containing particulate matter, were filtered through a

glass fibre paper (GF 52 Ø 110 mm, Schleicher & Schuell, Dassel, Germany) prior to extraction. Depending on the aqueous matrix, different volumes of water were extracted. In case of surface and sewage water, 50 mL water was extracted, while 250 mL was used for seawater samples. The 13C-labelled internal standards were supplemented to every sample prior to extraction to a final concentration of 100 ng L^{-1}. Solid phase extraction was carried out using OASIS HLB cartridges (6 cc, 200 mg, Waters, Milford, MA). The cartridges were pre-conditioned with 2 mL methanol and 2 mL Biosolve water. After loading, the cartridges were rinsed with 2 mL Biosolve water for surface and sewage water. For seawater, 3 x 2 mL Biosolve water was applied. Subsequently, the cartridges were dried under vacuum for 10 minutes. Elution was achieved using 2 x 2 mL methanol. Next, extracts were concentrated to 0.5 mL under a gentle stream of nitrogen. Finally, 0.5 mL of 2.5 mM ammonium acetate in water was added before transfer to LC-MS vials. Samples were stored at 4°C before analysis.

The LC-apparatus comprised of a 1200 series binary gradient pump and a 1100 series autosampler (Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was achieved using a Luna® C18 (2) HPLC column (5 μm particle size, 250 × 2.0 mm; Phenomenex Inc., Utrecht). 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, increasing to 90 % B in 10 min. This ratio was kept for 6 minutes before reversion to the initial conditions.

Analytes were detected with a Time-of-Flight mass spectrometer (ToF-MS) equipped with a dual electrospray ionisation interface (ESI MSD TOF, Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was operated in the negative ion mode. Instrument parameters were: drying gas temperature of 325°C, drying gas flow of 5 L min^{-1}, nebuliser pressure of 20 psi, capillary voltage of 3500 V, and chamber voltage of 3000 V. Before analyzing a series of samples, the ToF-MS apparatus was tuned and calibrated using the ESI Tuning Mix (Agilent Technologies, Santa Clara, CA, USA). During analysis, a reference solution was pumped into the MS-system at a rate of 50 μL min^{-1} using a separate sprayer connected to a 1100 series pump (Hewlett Packard, Palo Alto, CA, USA). This reference solution consisted of purine with a m/z ratio of 119.0363 and HP-0921 (hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine) with a m/z ratio of 980.0164 in ACN/H20 (95/5) (Agilent Technologies, Santa Clara, CA, USA). Accurate mass measurements could only be achieved if these reference masses were detectable. Chromatograms and spectra were recorded and processed using Agilent MassHunter Quantitative Analysis® software (Agilent Technologies, Santa Clara, CA, USA).

To demonstrate the applicability of this analytical approach, a validation study was carried out. Besides the validation procedure of the accredited lab of the Flemish Environment Agency (FEA), the SANCO/2007/3131 document (2007) was also used as guideline for the validation of this new analytical method. Validation of this analytical method in seawater resulted in limits of quantification (LOQs) varying from 2 to 200 ng L^{-1}, satisfying recoveries (92-134 %), and good linearity ($R^2 \geq 0.99$ for most analytes). The obtained RSD values indicated satisfying precision for all analytes in the different matrices (% RSD ≤ 20) (Table II).

Table II. Validation results: limits of detection (LODs) and quantification (LOQs) in ng L^{-1}, correlation coefficients (R^2), recoveries (%), and intra-laboratory reproducibility (R.S.D. %) of the targeted PFCs in sea water.

Compound	LOD	LOQ	R^2	Recovery	R.S.D.
PFBS	20	40	0.99	100	6
PFHxS	1	2	0.99	108	7
PFOS	1	2	0.99	101	7
PFDS	10	20	0.99	96	18
PFPA	50	100	0.99	97	7
PFHxA	20	40	0.99	103	12
PFHpA	10	20	0.99	101	8
PFOA	5	10	0.99	99	10
PFNA	10	20	0.99	102	11
PFDA	10	20	0.99	103	11
PFUnA	10	20	0.99	103	17
PFDoA	80	160	0.98	104	19
PFTeA	100	200	0.96	100	18
PFOSA	5	10	0.99	98	7

2.1.2.3. Detection of pesticides, PFCs and pharmaceuticals in biota

The analytical method for pharmaceutical analysis included 11 compounds. Paracetamol (99%), ketoprofen (99%), carbamazepine (>99%), diclofenac (>99%), salicylic acid (>99%), clofibric acid (97%), atenolol (≥98%), trimethoprim (≥98%), and chloramphenicol (≥99%) were purchased from Sigma-Aldrich (St-Louis, MO, USA). Ofloxacin (>99%) was obtained from ICN Biomedicals Inc. (Ohio, USA), while propranolol (>99%) was purchased from Eurogenerics (Brussel, Belgium). Next to the synthetic isobutcar 61 (4-3(isobutylamino-2-hydroxy-propoxy)carbazole), two deuterated pharmaceuticals were used as internal standard: atenolol-d7 (≥95%) and salicylic acid-d6 (≥98%) from Sigma-Aldrich (St-Louis, MO, USA).

Fourteen pesticides were included in this study. Dichlorvos (>98%), dimethoate (>99%), diazinon (>98%), pirimicarb (≥99%), linuron (>99%), metolachlor (≥98%), chloridazon (≥99%), chlorpyriphos (>99%), simazine (>99%), isoproturon (>99%),

terbutylazine (>98%), and diuron (>99%) were obtained from Sigma-Aldrich (St-Louis, MO, USA), while atrazine (>99%) and kepone (≥98%) were purchased from Chem Service (West Hester, PA, USA). Isoproturon-d6 (>99%) and atrazine-d5 (>99%) from Sigma-Aldrich (St-Louis, MO, USA) were used as internal standards.

Ten PFCs were examined in this study: four perfluorosulfonates (potassium perfluoro-1-butane sulfonate or PFBS, sodium perfluoro-1-hexane sulfonate or PFHxS, sodium perfluoro-1-octane sulfonate or PFOS, and sodium perfluoro-1-decane sulfonate or PFDS) and six perfluorocarboxylates (perfluoro-n-heptanoic acid or PFHpA, perfluoro-n-octanoic acid or PFOA, perfluoro-n-nonanoic acid or PFNA, perfluoro-n-decanoic acid or PFDA, perfluoro-n-undecanoic acid or PFUnA, and perfluoro-n-dodecanoic acid or PFDoA). All analytical PFC-standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada) with chemical purities of more than 98%. Five 13C-labelled internal standards were used as well: sodium perfluoro-1-[1,2,3,4-13C4]octane sulfonate, perfluoro-n-[1,2,3,4-13C4]octanoic acid, perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid, perfluoro-n-[1,2-13C2]decanoic acid, and perfluoro-n-[1,2-13C2]dodecanoic acid.

Reagents where of analytical grade when used for extraction and purification purposes, and of Optima® LC-MS grade for U-HPLC-MS/MS analysis. They were obtained from VWR International (Merck, Darmstadt, Germany) and Fisher Scientific UK (Loughborough, UK), respectively. For LC-ToF-MS analysis, methanol was purchased from Rathburn Chemicals (LTd Walkerburn, Scotland), while HPLC-grade water was obtained from Biosolve (Biosolve Chemicals, The Netherlands). Ammonium acetate (2.5 mM) in water was obtained through dilution of LC-MS Chromasolv® water containing 0.1% ammonium acetate (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany). Aqueous ammonium carbonate (Merck, Darmstadt, Germany) (2 mM) and aqueous formic acid (Merck, Darmstadt, Germany) (0.08%) were prepared by appropriate solution or dilution in ultra-pure water. Ultra-pure water was produced with an Arium 611 UV system (Sartorius Stedim Biotech, Aubagne, France).
Primary stock solutions of the pharmaceuticals and pesticides were prepared in ethanol at a concentration of 1 ng μL^{-1}, while methanol was used for the PFCs. Working standard mixture solutions were prepared by appropriate dilution of the stock solutions in ethanol and methanol, respectively. All solutions were stored at -20°C in the dark.

The sample preparation for pharmaceuticals consisted of a pressurized liquid extraction (PLE), which was performed on a Dionex ASE® 350 Accelerated Extractor with Solvent Controller (Dionex corp., Sunnyvale, CA, USA). A cellulose filter (27 mm, Dionex

corp.) was placed on the bottom of a 22-mL stainless steel extraction cell. Each cell was filled with 9.5 g of aluminium oxide 90 aktiv neutral (Dionex corp.). A mixture of 1 g of freeze-dried biotic sample with 1.5 g of diatomaceous earth (DE, ASE® Prep Diatomaceous Earth, Dionex corp.) was placed on top of the aluminium oxide. The internal standards were supplemented prior to extraction to a final concentration of 200 ng g^{-1}. A combination of acetonitrile/water (3/1) with 1% formic acid was used as extraction solvent. Extraction was carried out at 100°C during 3 cycles of each 10 min. The obtained ASE-extract (± 45 mL) was evaporated under nitrogen at 55°C to a final volume of 5 mL and further diluted to 100 mL with ultra-pure water. Next, solid phase extraction (SPE) was carried out using Strata-X cartridges (6 mL, 200 mg, Phenomenex B.V., Utrecht, Netherlands). The cartridges were preconditioned with 5 mL of methanol and 5 mL of ultra-pure water. After loading, the cartridges were rinsed with 5 mL of ultra-pure water. Elution was performed using 2 x 3 mL of methanol. Finally, this elute was evaporated under nitrogen at 55°C and reconstituted in 50 μL of acetonitrile with formic acid (0.08%) and 250 μL aqueous formic acid (0.08%). Prior to U-HPLC-MS-analysis, the extracts were centrifuged at 9000 rpm for 10 min at 4°C.

A similar combination of PLE and SPE was optimized for pesticide extraction and clean-up from biotic samples. The following differences may however be listed. The internal standards were spiked at a concentration of 100 ng g^{-1}. Each extraction cell was filled with 0.25 g of sample, 2 g of aluminium oxide, and 4.5 g of diatomaceous earth. Extraction was performed using a combination of acetonitrile/methanol (1/1), at 100°C during 3 cycle times of 3 min. The obtained ASE-extract was evaporated to 0.5 mL before dissolving it in 10 mL of ultra-pure water. SPE was carried out using Isolute ENV+ cartridges (10 mL, 200 mg, Biotage, Uppsala, Sweden). The cartridges were preconditioned with 5 mL of methanol and 5 mL of ultra-pure water with methanol (5%). Elution was performed using 5 mL of methanol and acetonitrile. Next, the elute was evaporated under nitrogen at 55°C to dryness and reconstituted in 50 μL methanol and 150 μL of 2 mM aqueous ammonium carbonate. After centrifugation, the elute was filtered using a Syringe-Driven Filter Unit of 0.22 μm (Millipore, Carritwohill, Cork, Ireland).

For analysis of PFCs in biotic samples, 1 g of freeze-dried sample, spiked with the C13-labelled internal standards at a concentration of 50 ng g^{-1}, was extracted with 10 mL of acetonitrile by homogenization with an Ultra-Turrax dispersing unit (Ika, Staufen, Germany). After centrifugation at 5000 rpm for 20 min at 4°C, the supernatans was reduced to 5 mL by evaporation under nitrogen at 55°C and subsequently diluted to 100 mL with ultra-pure water. Next, SPE was carried out using OASIS HLB cartridges (6

mL, 200 mg, Waters, Milford, MA). The cartridges were preconditioned with 2 mL of methanol and 2 mL of ultra-pure water. After loading, the cartridges were rinsed with 5 mL of ultra-pure water. Elution was performed using 3 x 2 mL of methanol and the elutes were centrifuged at 9000 rpm for 10 min at 4°C. Next, the elutes were evaporated to dryness under nitrogen at 55°C. Finally, 0.25 mL of methanol and 0.25 mL of 2.5 mM aqueous ammonium acetate were added before transfer to LC–MS vials. Samples were stored at -20°C before analysis.

For both the pesticides and pharmaceuticals, chromatographic separation was carried out using ultra-high performance liquid chromatography (U-HPLC). The apparatus comprised of an AccelaTM High Speed LC and an AccelaTM autosampler (Thermo Scientific, San Jose, CA, USA). Chromatographic separation was achieved using a Nucleodur C18 Pyramid U-HPLC column (1.8 μm, 100 x 2 mm, Macherey-Nagel, Düren, Germany). For the pharmaceuticals, the mobile phase constituted of 0.08% aqueous formic acid (A), 0.08% formic acid in acetonitrile (B), and isopropanol (C). A linear gradient was used starting from 98% A and 2% B, which was held for 0.8 min. The percentage of acetonitrile was increased to 65% B in 30 sec, further to 100% B in 1 min and held during 4 min. Next, 90% B and 10% C were applied to the column for 2 min, before equilibration at initial conditions for 2 min. Pesticide separation was achieved using methanol (D) and aqueous ammonium carbonate (2 mM) (E). The linear gradient started with a mixture of 98% E and 2% D for 1 min. The methanol percentage increased to 90% in 30 sec, and further to 100% in 3 min. Between samples the column was allowed to equilibrate at initial conditions for 1 min.
For PFC-analysis, the LC-apparatus comprised of a 1200 series binary gradient pump and a 1100 series autosampler (Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was achieved using a Luna® C18 (2) HPLC column (5 μm particle size, 250 × 2.0 mm; Phenomenex Inc., Utrecht). The mobile phase consisted of a mixture of (F) 2.5 mM aqueous ammonium acetate and (G) methanol. A linear gradient of 0.3 mL min^{-1} was used starting with a mixture of 50% F and 50% G, increasing to 90% G in 10 min. This ratio was kept for 6 min before reversion to the initial conditions.

Detection of pharmaceuticals and pesticides was carried out using a TSQ Vantage Triple Stage Quadrupole Mass Spectrometer (Thermo Electron) equipped with a heated electrospray ionization probe (HESI-II). The parameters as presented in Table 1 were found to be the optimal ionization source working parameters for the respective analytes. The mass resolution at the first (Q1) and third (Q3) quadrupole was set to 0.7 Da at full width at half maximum (FWHM). The cycle time was adjusted to 0.5 and 0.9

sec for pharmaceutical and pesticide analysis, respectively. The collision gas pressure was set at 1.5 mTorr and the chrom filter peak width at 10 sec.

Perfluorinated compounds were detected with a Time-of-Flight mass spectrometer equipped with a dual electrospray ionization interface (ESI MSD TOF, Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was operated in the negative ion mode. Instrument parameters were: drying gas temperature of 325°C, drying gas flow of 5 L min^{-1}, nebuliser pressure of 20 psi, capillary voltage of 3500 V, and chamber voltage of 3000 V. Before analyzing a series of samples, the ToF-MS apparatus was tuned and calibrated using the ESI Tuning Mix (Agilent Technologies, Santa Clara, CA, USA). During analysis, a reference solution was pumped into the MS-system at a rate of 50 μL min^{-1} using a separate sprayer connected to a 1100 series pump (Hewlett Packard, Palo Alto, CA, USA). This reference solution consisted of purine with a m/z ratio of 119.0363 and HP-0921 (hexakis(1H,1H,3H-tetrafluoropropoxy)-phosphazine) with a m/z ratio of 980.0164 in ACN/H20 (95/5) (Agilent Technologies, Santa Clara, CA, USA). Accurate mass measurements could only be achieved if these reference masses were clearly present. Chromatograms and spectra were recorded and processed using Agilent MassHunter Quantitative Analysis® software (Agilent Technologies, Santa Clara, CA, USA).

The target analytes were identified based on their retention time relative to that of the internal standards. For pharmaceutical and pesticide analysis, using U-HPLC-QqQ-MS/MS in the selected reaction monitoring mode (SRM), at least two transitions were monitored. The relative abundances of these specific transitions were compared with those of the standards and both product ions were used for quantification purposes. Identification of the PFCs, using LC-ToF-MS, was performed on the base of their accurate mass. Within this study, a maximum mass error of 10 ppm was allowed (Sinclair et al., 2006).

For pharmaceutical and pesticide analysis, using U-HPLC-QqQ-MS/MS in the selected reaction monitoring mode (SRM), at least two transitions were monitored. The MS/MS transitions (at least two), S-lens voltages, and collision energies were optimized for each individual compound (Table III).

Identification of the PFCs, using LC-ToF-MS, was performed on the base of their accurate mass. Within this study, a maximum mass error of 10 ppm was allowed (Taniyasu et al., 2005). The theoretical masses, the mean measured masses, and the obtained mass errors are presented in Table IV.

The method performance of each of the three newly optimized analytical procedures was determined by constructing seven-point calibration curves in tissue matrix. To this end, freeze-dried M. edulis samples were used. The method proved to be applicable to pacific oysters (*C. gigas*) and brown shrimps (*C. crangon*) as well (data not shown). Good linearities were obtained for all analytes (regression coefficients ≥0.99), except for chlorpyriphos for which R^2 equalled 0.98. As can be deduced from Table III and Table IV, all obtained recoveries were between 90 and 106%, indicating good accuracy for all compounds. The obtained LOQs varied between 0.1 and 10 ng g^{-1}, indicating a good sensitivity of the reported methodologies.

Table III. SRM transitions, MS parameters, recoveries, and limits of quantification (LOQs) of the targeted pharmaceuticals and pesticides in *Mytilus edulis* extracts (n = 21).

Compound	tR (min)	Precursor ion (m/z)	Product ions (m/z)	S-lens (V)	Collision E (eV)	Recovery (%) (x ± RSD)	LOQ ng g^{-1}
Pharmaceuticals							
Atenolol	0.83	267.1 (+)	190.1, 145.0	102	18, 26	97 ± 13	1
Ofloxacin	2.35	362.1 (+)	318.2, 261.1	176	18, 27	102 ± 14	5
Propranolol	2.35	260.2 (+)	183.1, 116.1	138	18, 17	98 ± 13	1
Trimethoprim	2.25	291.1 (+)	261.1, 230.1	188	25, 23	101 ± 13	1
Paracetamol	2.15	152.0 (+)	110.1, 65.1	52	16, 30	97 ± 26	2.5
Salicylic acid	2.85	137.0 (-)	93.1, 65.1	51	20, 32	103 ± 10	10
Clofibric acid	3.46	213.0 (-)	127.1, 85.1	73	19, 13	100 ± 20	1
Chloramphenicol	2.58	321.0 (-)	257.1, 152.1	104	15, 19	95 ± 15	2.5
Ketoprofen	3.19	255.0 (+)	209.2, 105.0	295	14, 24	100 ± 12	5
Carbamazepine	2.78	237.1 (+)	194.1, 193.1	93	19, 33	100 ± 11	1
Diclofenac	3.55	296.0 (+)	250.1, 214.1	78	13, 34	98 ± 16	2.5
Atenolol-d7	0.96	274.1 (+)	190.1, 145.0	111	19, 27	-	-
Salicylic acid-d6	2.84	141.1 (-)	97.1, 69.2	52	19, 33	-	-
Isobutcar 61	2.36	313.8 (+)	222.1, 130.1	170	19, 20	-	-
Pesticides							
Atrazine	3.25	216.1 (+)	174.1, 68.1	83	17, 36	95 ± 19	1
Diuron	3.25	233.0 (+)	72.1, 46.2	71	18, 16	103 ± 8	1
Isoproturon	3.21	207.1 (+)	72.1, 46.2	78	19, 17	102 ± 8	1
Kepone	4.30	506.6 (-)	426.8, 424.8	157	21, 20	99 ± 19	1
Simazine	3.11	202.1 (+)	132.1, 124.1	77	18, 18	100 ± 8	5
Terbutylazine	3.41	230.2 (+)	174.1, 104.1	70	18, 33	100 ± 8	1
Dichlorvos	3.23	221.0 (+)	109.1, 79.1	81	19, 28	100 ± 10	1
Dimethoate	2.87	230.0 (+)	199.0, 79.1	63	10, 34	104 ± 10	10

Diazinon	3.80	305.1 (+)	169.1, 97.0	87	20, 34	104 ± 17	1
Pirimicarb	3.20	239.1 (+)	182.2, 72.2	74	15, 33	101 ± 10	1
Linuron	3.36	249.0 (+)	182.1, 160.1	83	16, 18	105 ± 11	1
Metolachlor	3.61	284.1 (+)	252.2, 176.2	69	15, 24	101 ± 11	1
Chloridazon	2.90	222.0 (+)	104.1, 77.1	95	23, 36	104 ± 9	1
Chlorpyriphos	4.64	349.8 (+)	199.9, 197.9	82	22, 21	98 ± 17	1
Atrazine-d5	3.23	221.1 (+)	179.1, 101.1	79	19, 27	-	-
Isoproturon-d6	3.21	213.1 (+)	78.2, 52.2	69	20, 19	-	-

Table IV. Characteristics of the PFC-analysis using ToF-MS: theoretical masses, mean measured masses, mean mass errors, internal standards, recoveries, and limits of quantification (LOQs).

Compound	Theoretical m/z [M-H]- ion	Mean measured m/z	Mean mass error (ppm)	Internal standard	Recovery (%) (x + RSD)	LOQ ng g^{-1}
PFHpA	362.9696	362.9705	3.2	13C4 PFOA	103 ± 10	2
PFOA	412.9664	412.9680	4.1	13C4 PFOA	100 ± 9	1
PFNA	462.9632	462.9648	4.3	13C5PFNA	101 ± 15	2
PFDA	512.9600	512.9625	4.9	13C2PFDA	105 ± 15	2
PFUnA	562.9563	562.9581	4.9	13C2PFDA	98 ± 15	2
PFDoA	612.9531	612.9565	5.5	13C2PFDoA	90 ± 17	5
PFBS	298.9430	298.9436	2.9	13C4PFOS	94 ± 18	5
PFHxS	398.9366	398.9387	4.9	13C4PFOS	106 ± 23	0.1
PFOS	498.9302	498.9317	3.9	13C4PFOS	100 ± 16	0.1
PFDS	598.9233	598.9250	3.7	13C4PFOS	96 ± 16	0.1

2.1.2.4 Analysis of PAHs, PCBs, PBDEs and OCPs in biota

Biota samples were prepared as mentioned in § 0. About 3g of freeze-dried mussel or oyster tissue, 0.5g of liver or 7g of fish filet was taken as sample intake for a pressurized liquid extraction (PLE), which was performed on a Dionex ASE® 350 Accelerated Extractor with Solvent Controller (Dionex corp., Sunnyvale, CA, USA). A cellulose filter (27 mm, Dionex corp.) was placed on the bottom of a 22-mL stainless steel extraction cell. Each cell was filled with 5g of C18 (Bakerbond ® Octadecyl 40μm PrepLC Packing, Mallinckrodt Baker, Phillipsburg, USA) followed by 17g of basic aluminium oxide (AlOx) (J.T.Baker, Phillipsburg, USA) and Hydromatrix (Varian inc., UK) to fill the void volume of the cells. In the case of fish filet, only 10 g of AlOx was used. The internal standards were supplemented prior to extraction to a final concentration of 100 ng g^{-1}. Dichloromethane (HPLC grade, Chemicals Walkerburn, Scotland) was used as extraction solvent. Extraction was carried out at 100°C during 3 cycles of each 10 min,

with a purge time of 120 s and flush volume of 60%. The obtained ASE-extract (± 45 mL) was concentrated to 1 ml prior to gel permeation chromatography (GPC) using an evaporative solvent reduction apparatus (Zymark Turbovap ® II; Caliper, Hopkinton, MA, USA) and filtered with 13 mm Acrodisc® syringe filters with 0,2μm GHP Membrane (Pall GmbH). GPC was performed with an ASPEC XL system (Gilson, Villiers le Bel, France) with a modified injection and equipped with UV-detector. The GPC columns used were a guard-column (Waters Corporation, Milford, MA, USA) (4.6x13mm) in series with two Envirogel™ columns (Waters Corporation, Milford, MA, USA) (19x150mm and 19x300mm). 1 ml of ASE extract was injected in the GPC, the mobile phase was dichloromethane with a flow rate of 5 ml/min. The extract was reduced with the Zymark Turbovap® II to 1 ml, the recipient was rinsed with 5 ml hexane:iso-octane (9:1, *v*/*v*) and further reduced. Recovery standards were added, used to determine the recoveries of internal standards for QA/QC purposes.

The extracts were injected by LV injection (70μL) in a BEST PTV Injector (Thermo Electron Corporation, Austin, TX, USA) and further analysed on GC-MS as described in § 0.

Multi-level calibration curves (r2 > 0.995) in the linear response interval of the detector were created for quantification. The identification was based on retention times, ion chromatograms and intensity ratios of the monitored ions for quantification. The quality control was performed by regular analysis of procedural blancs, internal reference material (mussel) and procedural spikes of 100 ng/g. The limits of quantification ranged between 1 and 20 ng g^{-1} DW.

2.1.3. Chemical analysis: summary of existing techniques

2.1.3.1. Analysis of organotin compounds, PAHs, PCBs, PBDEs and OCPs in water, sediment, SPM and biota

Sediment samples were centrifuged to obtain the clay fraction (<63 μm) 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.

For analysis of PAHs, PCBs, PBDEs and OCPs a single extract method is used. 1 to 5 L water samples were extracted using solid-phase extraction with Bakerbond Speedisk C18 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).

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).

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. Quantification limits were overall 1 ng g^{-1} DW, for OCPs 1 to 20 ng g^{-1} DW.

2.1.3.2. Analysis of 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 $\mu g.L^{-1}$.

2.1.3.3. Analysis of 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).

2.1.3.4. Analysis of 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.

2.1.3.5. Analysis of Lead and Cadmium in marine sediments

10g of sediment was dried at a temperature of 105°C until a constant weight was achieved. The dried sediment was subsequently grinded followed by digestion. For the

latter, 1g of dried sediment was transferred to an acid-cleaned conical flask of 100 mL followed by addition of 7.5 mL nitric acid (69%, AnalaR NORMAPUR, VWR) and 2 mL of hydrochloric acid (37%, AnalaR NORMAPUR, VWR). The flasks were covered with a watch glass and subsequently heated for 2 hours at 110°C. After cooling, the samples were poured over a paper filter and the filtrate was recovered in an acid-cleaned volumetric flask of 100 mL. The filtrate was then diluted up to 100 mL with deionized water. Samples were analyzed by graphite furnace atomic absorption spectrometry (GF-AAS) with Zeeman background correction on a Thermo Scientific iCE 3000. Samples were further diluted 10 times with deionized water and a sample volume of 15 μL was used. As a modifier, $MgNO_3$ was used. The ashing temperatures for analysis of Pb and Cd were set at 650 and 450°C and the atomization temperatures at 1200 and 1500°C, respectively. Pb was measured at a wavelength of 283.3 nm, Cd at 228.89 nm. The detection limits of Pb and Cd were 0.5 and 0.1 $\mu g\ g^{-1}$ DW, respectively.

2.1.3.6. Analysis of PCBs and OCPs in tern eggs

Eggs were stored at -18°C. Total egg weight (g), length, width (mm) and shell thickness (μm) were recorded. The egg's content was freeze-dried with a Christ LMC-2 (Osterode, Germany) and homogenized with a Fritsch Pulverisette (Idar-Oberstein, Germany). Dry weight and fat weight of the egg was determined. Samples were stored at -18°C prior to further analysis.

An accurately weighed aliquot (0.2 – 0.3 g) of the freeze-dried sample was taken for analysis. The sample was extracted with the ASE as described in 2.1.2.4. As internal standard CB155 was added before extraction. Consequently a column was prepared with 0.4g of acidified silicagel. The silicagel was made by adding 9g sulphuric acid (95-97%, pro-analyse, Darmstadt, Germany) to 12g of silica gel (Baker analyzed, 0.063-0.200mm, Mallinckrodt Baker B.V., Deventer, Holland). The column was precleaned with elution of 10 ml hexane. The extract was brought on the column and eluted with 20 ml of hexane (Baker ultra-resi-analyzed, Mallinckrodt Baker B.V., Deventer, Holland). After adding recovery standards CB29 and CB129 and 100μL of iso-octane, the eluate was further reduced with a nitrogen flow with the Zymark Turbovap® II (Capiler, Hopkinton, MA, USA) to 1ml. The extract was analysed for 14 PCB congeners (CB-18,-31,-28,-52,-44,-101,-118,-53,-105,-138,-187,-156,-180,-170) and 10 organo-chlorinated pesticides (hexachloorbutadieen, hexachloorbenzeen, gammahexachloor-cyclohexaan, beta-hexachloorcyclohexaan, heptachlor, chlordane-trans and cis, pp'-DDE, op'-DDT, pp'-DDT) with the method as described in 2.1.2.4. Recoveries for PCBs and OCPs respectively were between 97 and 132% and 91 and 130%. The quantification limit for PCBs in eggs was 1 $ng\ g^{-1}$ DW for OCPs 5 $ng\ g^{-1}$ DW.

2.2 Ecotoxicological assessment

2.2.1. 72h growth inhibition test with *Phaeodactylum tricornutum*

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.

2.2.2. 24h larval development test with Crassostrea gigas

The *C. 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^{-1}. 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 50% effect concentration (EC50) was calculated using the trimmed Spearman-Karber method (Hamilton *et al.*, 1977).

2.2.3. Biomarker experiments with *Mytilus edulis*

2.2.3.1 General description

An *in situ* biomarker experiment with the blue mussel *Mytilus edulis* was conducted in the harbours of Nieuwpoort (NP2), Oostende (OO2) and Zeebrugge (ZB1, ZB2). *Mytilus edulis* was collected at the Eastern Scheldt for the experiment in the autumn of 2008 and at the aquaculture site of Reynaert & Versluys (W03') (which also functioned as control station) for the experiment in the spring of 2009 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 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 six weeks. Abiotic parameters (salinity, temperature, oxygen concentration, chlorophyll a and suspended particulate matter) were recorded three times during the course of the experiment. After recollection, the organisms were taken to 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.

2.2.3.2 Cellular energy allocation

The goal of the cellular energy allocation assay (CEA) is to determine the available energy (E_a = protein + carbohydrate + lipid content) and the energy consumption (E_c, 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 *M. 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.

Eventually, the available energy is divided by the energy consumption, yielding the CEA value in hours. This can be interpreted as the theoretical period of time that the organism can survive without food at that rate of energy consumption.

2.2.3.3 Lysosomal membrane stability

To determine the lysosomal membrane stability (LMS) the mussel valves were prised apart gently to drain off excess water and withdraw 0.1 ml of haemolymph from the posterior adductor muscle using a 1 ml hypodermic syringe containing 0.1 ml physiological saline. The physiological saline solution contains 4.77 g Hepes, 25.48 g Sodium chloride, 13.06 g Magnesium sulphate, 0.75 g Potassium chloride, 1.47 g Calcium chloride and is made up to one litre and adjusted to 7.36 pH using 1M NaOH. 50 μl of the haemolymph and physiological saline solution is pipetted onto a slide and incubated in a light-proof chamber. After 15 to 20 minutes excess suspension is drained off and 40 μl Neutral Red (NR) working solution is pipetted onto the haemocytes. The NR stock solution contains 28.8 mg NR dye powder and 1ml DMSO, the working

solution is prepared by mixing 10 µl NR stock solution with 5 ml physiological saline and lasts 4 hours. After 15, 30, 60, 90, 120 and 180 minute incubation periods the haemocytes are examined under the microscope for abnormalities, enlargement and cell leakage. The NR retention time or endpoint is reached when 50% or more cells show enlargement or cell leakage.

2.2.3.4 EROD activity

For the analysis of EROD (ethoxyresorufin-O-deethylase) 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 ethoxyresorufin 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.

2.2.3.5 Alkali-labile phosphates

For the alkali-labile phosphate (ALP) method the gonads were dissected, one half was used for protein and the other for phosphate analysis. The protein concentration was determined using the Bradford method (Bradford, 1976). For phosphate analysis the gonad was homogenised in 1 ml of 25mM Hepes-NaOH buffer with pH 7.4 (containing 125 mM NaCl, 1 mM dithiothreitol and 1mM EDTA). The homogenate was centrifuged at 10.000 g for 15 minutes at 4°C. Subsequently, the supernatant was removed and 500 µl was mixed with 350 µl acetone (35%), this solution was centrifuged at 10.000 g for 5 minutes at 4°C. After centrifugation, the obtained pellet was dissolved in 200 µl 1M NaOH at 70°C in a water shaking bath for 30 minutes with intermediate vortexing. To determine the levels of inorganic free phosphates 75 µl of the sample was mixed with 125 µl trichloroacetic acid, 630 µl ultrapure water, 170 µl molybdenum reagent (containing ammonium molybdate tetrahydrated and H2SO4) and 50 µl Fiske-subbarow reducer (Sigma, St. Louis, MO). The solution was incubated for 10 minutes after which

the absorbance was measured at a wavelength of 660 nm using a spectrophotometer. ALP levels were determined by the μg phosphates/ mg protein ratio and μg phosphates / g ww of the gonad.

2.3. Ecological assessment

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.

Epibenthic, macrobenthic and hyperbenthic fauna were sampled in order to assess the density and diversity of the different ecological compartments of the benthos in relation with a possible contamination gradient from the inner harbours to the open sea.

2.3.1 Macrobenthos biodiversity

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. Brown shrimps (*Crangon crangon*) and liver of flatfishes (*Limanda limanda*, *Solea solea* and *Pleuronectes platessa*) 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 cannot 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.

2.3.2 Long term cage experiments with Mytilus edulis and Crassostrea gigas

Cage experiments with transplanted blue mussels (*M. edulis*) and pacific oysters (*C. 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.

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 were 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 (SLI) 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 (CI), 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 were collected (same sampling frequency as above) 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 shell length (mm) was also determined.

Water temperature (°C), salinity (PSU), oxygen content (mg L^{-1}), chlorophyll a concentration (μg 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).

2.3.3 Population characteristics of the common tern Sterna hirundo

Subcontractant INBO sampled ten eggs of the common tern (*Sterna hirundo*) during spring 2007, 2008 and 2010 from a breeding colony in the harbour of Zeebrugge. The eggs were analyzed for pollutants. Population data of the reproductive success and the development of the chicks originating from that common tern colony were recorded.

2.3.4 Population characteristics of the mysid shrimp Neomysis integer

In the laboratory all mysid shrimps were sorted out, identified to species level and counted. Per species all individuals were selected, sexed and categorized into six life cycle stages according to Mauchline (1980): adult males, adult females, subadult males, subadult females, juveniles and gravid females. Embryo counts (i.e. brood sizes) were made on all gravid females with a completely full marsupium, present in the subsample.

2.4. Novel approach: passive sampling

2.4.1. Monitoring freely dissolved, time-weighted average concentrations of contaminants

Polydimethylsiloxane sheets were retained as passive samplers based on the properties of the material (Rusina et al., 2007) (55mm x 90mm x 0.5mm) (AlteSil Laboratory Sheet, Altec, Bude, England). Before use the sheets were pre-extracted for 100 hours with ethylacetate. Subsequently the sheets were spiked with performance reference compounds (PRCs). The method of spiking is described in Booij *et al*. (2002). Then the sheets were 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 to 10 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 2.1.3. Concentrations could be expressed in water concentrations using diffusion and kinetic models (Huckins *et al.*, 2006; Rusina *et al.*, 2010).

2.4.2. Passive samplers as a surrogate for biota in bioaccumulation assessment

POP's have the ability to accumulate in the lipids of fish and shellfish (i.e. they bio-accumulate). A passive sampler can mimic this bioaccumulation. It is suggested that passive samplers can therefore be used to predict concentrations of these contaminants in both water and mussels (Booij et al, 2006). Two experiments were performed during this project where mussels and passive samplers were deployed simultaneously during autumn of 2008 and the spring of 2009. The cages were deployed for a 6-week period at 5 passive sampling stations (see Figures 1 and 2). After deployment the sheets as well as the mussels were recovered and analysed as described in § 2.1.6.1 and § 2.1.4.3 respectively. The mussels and sheets were analyzed for organic contaminants as described in § 2.1.2. and § 2.1.3.

2.4.3. Exposing organisms to realistic contaminant mixtures: passive dosing

2.4.3.1. Development

A new application developed in this project, aims to use passive samplers to administer chemical compounds to ecotoxicological test medium through the process of 'passive dosing'. 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 concentrations of the different compounds on the samplers were chosen to achieve a concentration of 1 μg L^{-1} in the test medium. 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 (μg L^{-1}), C_s is the concentration in the sampler (μg 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.

2.4.3.2. 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; samplers deployed during the sampling campaign of 2007 and during the biomarker experiments in 2008 and 2009 were used) were transferred to 50 mL of uncontaminated test medium in a 100 mL erlenmeyer flask. Prior to addition of the test organism, the erlenmeyer flasks 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. The test organisms used were *Phaeodactylum tricornutum* and *Crassostrea gigas*. The test protocols are described in §0 and §0.

2.4.3.3. Interpretation of mixture toxicity

The interpretation of mixture toxicity performed in this study was performed assuming dose-additivity for all compounds in the mixture. To this end, the toxic units (TUs) present at each sampling station were calculated for each compound by dividing the measured water concentration (Cw) by its EC50 value:

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

The hazard index (HI) of the mixture was then calculated as:

$$HI = \sum TU_i \quad (4)$$

with TU_i as the toxic unit of the i^{th} compound in the mixture.

As only PCBs and PAHs were measured in the passive samplers, measured environmental concentration data (i.e. obtained via conventional monitoring) were used rather than the actual testing concentrations achieved via passive dosing to calculate TU and HI. The mixture toxicity assessment was performed for the marine diatom *P. tricornutum*, as for the other test organism (*C. gigas*) the lack of available ecotoxicity data was too large. For *P. tricornutum*, data were scarce as well. For this reason, ecotoxicity data were also collected for other marine diatoms to fill data gaps. As such, this mixture assessment represents a low tier assessment. The ecotoxicity data are available on the restricted pages of the project website.

In a first assessment it became clear that HI was too low to explain the observed toxicity of the mixtures and that the observed differences between sampling stations could not be explained. This could partially be due to the fact that a single grab sample does not reflect the average concentration of contaminants as opposed to passive sampling. In order to obtain a better view of the potential mixture toxicity at each of the passive sampling stations, the minimum and maximum concentrations of each contaminant were collected per station. These values were then used in a Monte-Carlo analysis, in which 100,000 random concentration values were sampled (using latin-hypercube sampling) per compound and per station. A uniform distribution was chosen as insufficient monitoring data were available to obtain a reliable concentration distribution. For each iteration, the mixture HI was calculated. Finally, the distribution of the 100,000 HI values was explored.

2.4.4. Passive samplers as a central tool to monitor the distribution of pollutants in the marine environment – Equilibrium modeling

An equilibrium model has been developed – using the R statistical package – to model average environmental concentrations of micropollutants in SPM, sediment and biota based on freely dissolved, TWA concentrations derived from passive samplers.
Concentrations in sediment and SPM were calculated as follows:

$$C_{sol} = C_{diss} \cdot K_{oc} \cdot f_{oc,sol} \quad (5)$$

with C_{sol} as the concentration of contaminants in solids (i.e. sediment or SPM; μg kg^{-1}), C_{diss} as the freely dissolved concentration in seawater (μg L^{-1}) derived from passive sampling, K_{oc} as the organic carbon-water partitioning coefficient (L kg^{-1}) and $f_{oc,sol}$ as the fraction of organic carbon in the solids.
Concentrations in biota were calculated according to:

$$C_{org} = C_{diss} \cdot K_{ow} \quad (6)$$

with C_{org} as the concentration in organisms (i.e. shrimps, mussels, flatfish liver or flatfish tissue; μg kg^{-1}) and K_{ow} as the octanol-water partitioning coefficient (L kg^{-1}). K_{ow} and K_{oc} were retrieved from databases, $f_{oc,sol}$ were measured values from the SPM and sediment samples obtained in this study.
The modeled values were compared to the measured data corresponding to the time and place of the respective passive sampling campaigns. For the offshore passive sampling stations A2 and RV, median values of all offshore sampling stations were used for this comparison. To assess the model performance, the model bias MB was calculated:

$$MB = 10^{\frac{\sum_{i}^{n} \text{Predicted(i)}}{\sum_{i}^{n} \text{Observed(i)}}} \quad (7)$$

All modeling was performed using the free software R.

2.5. Integrated risk assessment and monitoring

2.5.1. Introduction

The objective of monitoring micro-contaminants is to assess the risk that they represent for the marine ecosystem and man, in the role of top predator. Monitoring programmes traditionally assess the status of the marine environment and changes that occur therein, due to natural causes and human interference. The latter being both the introduction of substances through human activities and the opposite through remedial actions. The classic approach has been to measure concentrations of substances in the different compartments and to compare these values against threshold or quality values. However, it was soon recognised that measuring concentrations alone does not suffice. Other substances may be present that are not part of the monitoring scheme or their

combined presence will cause effects that cannot be predicted by their individual concentrations. Looking at the effects directly has been introduced as a way to overcome this shortcoming. Yet, it is again impossible to consider and follow up on all possible effects. The health of the marine ecosystem needs also to be evaluated through the presence and the distribution of species. This clearly illustrates the need for a holistic approach or integrated monitoring. Large scale monitoring programmes such as OSPAR and HELCOM have introduced this as the ecosystem approach to monitoring.

The central assumption in our approach to integrated monitoring of micropollutants in the marine environment is, that these substances move from one compartment to another through the dissolved phase. The driving force is physico-chemical partitioning between the phases. If the properties of every phase or matrix were accurately known, one could predict the amount of any given substance in any given phase, based on the concentration of that substance in one of the phases. The latter is of course impossible as this information is not available. The properties of any given phase are generally not sufficiently known and, they tend to differ both in space and time. Introducing a reference phase with known properties i.e. the passive sampler (PS) of this study, overcomes this problem (see Figure 3).
Determining the concentration of a substance in a PS is relatively straightforward and this can be linked directly to the concentration in the freely dissolved phase. In other words, the contaminant pressure or the measure to which it will be distributed through the compartments of the ecosystem, can be determined. This is in fact an exposure assessment similar to the classical approach above but without the shortcomings caused by the natural variability of the different compartments. Furthermore, if the biomagnification potential of a substance is known, it is possible to assess the risks for top predators including man. As before, not every potential harmful substance will be determined in the sampler. Exposing test organisms to samplers is an elegant way to expose them to the conditions in the field without elaborate extraction procedures. They will essentially face the same substances as the organisms in the field. Hence, effects caused by unknown harmful substance will be observed. As before, this exposure and effect assessment needs to be backed up by an ecological evaluation of the situation in the field.

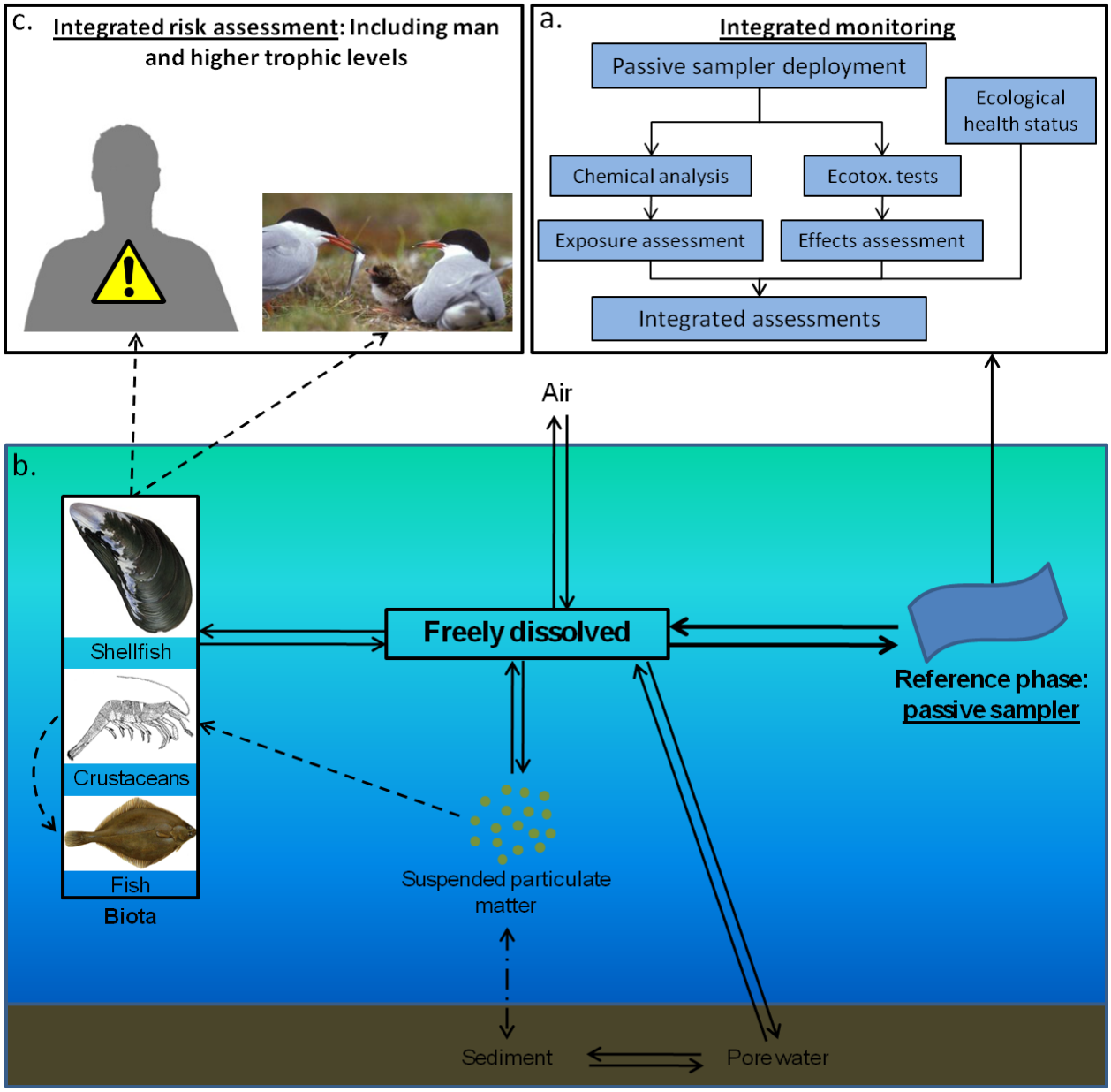

Figure 3. Schematic representation of the use of passive samplers as (a) a central tool for integrated monitoring and (b) a reference phase to estimate concentrations of micropollutants in the different compartments of the marine environment; (c) represents the resulting integrated risk assessment.

The challenge is therefore to evaluate if concentrations of substances (both known i.e. analysed and unknown i.e. through their effects) in the dissolved phase can be used to predict and/or assess the pressure to the ecosystems and by extension to man as a top predator. Ultimately this should lead to a scale indicating the pressure of contaminants in an environment as an element of a more holistic risk assessment.

2.5.2. Environmental risk assessment of single substances

The currently used internationally accepted risk and impact assessment procedures (e.g. REACH guidance documents) were employed for the environmental risk assessment of the micropollutants monitored in this study. For the risk assessment of single substances, a conventional approach was followed in which the (Predicted) Environmental Concentration ((P)EC) was compared to the Predicted No Effect Concentration (PNEC). A Risk Characterisation Ratio (RCR = (P)EC/PNEC) greater than 1 indicates a risk to the environment. A risk assessment was performed for the marine and sediment environment.

For the assessment of the risks, a comprehensive literature search was performed to obtain the following safety values for over 100 substances analyzed in this project: PNEC values of EU Risk Assessment Reports, EQS values of the WFD and EAC values of OSPAR. Safety values from other sources were also examined (i.e. pesticides databases and grey literature). The (P)ECs used in the risk assessment are measured values obtained from chemical analysis of the samples collected during the sampling campaigns of this project. As a novel approach, the concentration data (for PCBs and PAHs) obtained via equibrium modeling using passive sampling data were also used to perform a risk assessment. A comparison between the conventional approach and this novel approach is made.
The quality criteria used to assess the risks of micropollutants in the environment are summarised in Table V. Some PNEC values were calculated based on the available ecotoxicity data from literature, using the appropriate assessment factors according to the REACH regulation.

Table V. Quality criteria used for the risk assessment of micropollutants in the water and sediment compartment. AA-EQS: annual-average environmental quality standard; LL-EAC: lower-limit environmental assessment criterion; PNEC: predicted no-effect concentration.

	Water			Sediment		
Compound	Value (μg/L)	Type	Ref.[1]	Value (μg/kg DW)	Type	Ref.[1]
Acenaphthene	0.06	AA-EQS	a	0.16	PNEC	h
Acenaphthylene	4	AA-EQS	a	--	--	--
Anthracene	0.1	AA-EQS	b	50	LL-EAC	i
Benzo(a)anthracene	0.3	AA-EQS	a	100 (p)	LL-EAC	i
Benzo(a)pyrene	0.05	AA-EQS	b	100 (p)	LL-EAC	i
Benzo(b)fluoranthene	sum = 0.03	AA-EQS	b	140	PNEC	h
Benzo(k)fluoranthene				140	PNEC	h
Benzo(ghi)perylene	sum = 0.03	AA-EQS	b	84	PNEC	h
Indeno(1,2,3-cd)pyrene				63	PNEC	h
Benzo(e)pyrene	--	--	--	--	--	--
Chrysene	1	AA-EQS	a	100 (p)	LL-EAC	i

Dibenz(a,h)anthracene	0.5	AA-EQS	a	--	--	--
Fluoranthene	0.1	AA-EQS	b	500 (p)	LL-EAC	i
Fluorene	2	AA-EQS	a	--	--	--
Naphthalene	1.2	AA-EQS	b	50	LL-EAC	i
Perylene	--	--	--	--	--	--
Phenanthrene	0.1	AA-EQS	a	100	LL-EAC	i
Pyrene	0.04	AA-EQS	a	50 (p)	LL-EAC	i
sum7PCBs	0.002	AA-EQS	a	1 (p)	LL-EAC	i
TBT	0.0002	AA-EQS	b	0.005 (p)	LL-EAC	i
PFOS	2.5	PNEC	c	--	--	--
Salicylic acid	11.2	PNEC	d	--	--	--
Paracetamol	0.92	PNEC	d	--	--	--
Carbamazepine	0.25	PNEC	d	--	--	--
Atenolol	32	PNEC	d	--	--	--
Propranolol	0.01	PNEC	d	--	--	--
Bezafibrate	0.023	PNEC	d	--	--	--
Trimethoprim	4.8	PNEC	d	--	--	--
2,4,6-trichlorophenol	1.94	PNEC	d	--	--	--
2,4-dichlorophenol	0.06	PNEC	d	--	--	--
3-methylphenol	0.267	PNEC	d	--	--	--
4-chloro-3-methylphenol	0.0917	PNEC	d	--	--	--
4-chlorophenol	2.5	PNEC	d	--	--	--
Bisphenol A	0.15	PNEC	e	--	--	--
Dettol	0.036	PNEC	d	--	--	--
Nonylphenol	0.033	PNEC	f	--	--	--
Phenol	0.77	PNEC	g	--	--	--
Bis-(2-ethylhexyl)phthalate	0.62	PNEC	d	--	--	--
Dimethylphthalate	0.012[2]	PNEC	d	--	--	--
Butyl benzyl phthalate	0.17	PNEC	d	--	--	--
Diethyl phthalate	2.04	PNEC	d	--	--	--
Alachlor	0.3	AA-EQS	b	--	--	--
Diuron	0.2	AA-EQS	b	--	--	--
Simazine	1	AA-EQS	b	--	--	--
Isoproturon	0.3	AA-EQS	b	--	--	--
Atrazine	0.6	AA-EQS	b	--	--	--
Azoxystrobin	0.0049	PNEC	d	--	--	--
Carbendazim	0.0258	PNEC	d	--	--	--
Chloorbromuron	--	PNEC	d	--	--	--
Chloridazon	0.017[2]	PNEC	d	--	--	--
Chlortoluron	0.00085[2]	PNEC	d	--	--	--
Cyanazine	0.0048	PNEC	d	--	--	--
Desisopropylatrazine	--	PNEC	d	--	--	--
Flufenacet	0.00029	PNEC	d	--	--	--
Linuron	0.0024	PNEC	d	--	--	--
Metazachlor	0.00466[2]	PNEC	d	--	--	--
Methabenzthiazuron	0.0033[2]	PNEC	d	--	--	--
metobromuron	--	PNEC	d	--	--	--
metolachlor	0.01	PNEC	d	--	--	--
metoxuron	--	PNEC	d	--	--	--
monolinuron	0.0139	PNEC	d	--	--	--
pirimicarb	0.00065	PNEC	d	--	--	--

propachlor	0.00352	PNEC	d	--	--	--
terbutylazine	0.002	PNEC	d	--	--	--
sum6PBDEs	0.0002	AA-EQS	b	62 (p)	LL-EAC	i

[1]a. VR, 2010; b. EU, 2008; c. OSPAR, 2006; d. deducted using literature ecotoxicity data from literature and data generated in this study; e. ECB, 2003; f. ECB, 2002; g. ECB, 2006; h. CTPHT, 2008; i. OSPAR, 2004

[2] Based on an incomplete dataset, values should be used with caution.

2.5.3. Integrated risk assessment: risk to higher predators and man

To assess the risk of micropollutants in the marine environment to higher predators and man, various safety values were collected from different sources for the compounds analyzed in this project. The collected data are summarised in Table VI to VIII.

The quality criteria for secondary poisoning to predators (Table VI) were LL-EAC values and a PNECoral (PFOS). As such, an RCR was calculated in a similar as for water and sediment (§2.5.2) by dividing the concentration in bivalves (the (P)EC) by the LL-EAC or PNECoral.

Table VI. Quality criteria used for the assessment of secondary poisoning of predators by micropollutants. The quality criteria all apply to marine bivalves (mussels). Provisional values are indicated by '(p)'.

Compound	Value (µg/kg DW)	Type	Ref.[1]
Anthracene	5 (p)	LL-EAC	a
Benzo(a)pyrene	5000 (p)	LL-EAC	a
Fluoranthene	100 (p)	LL-EAC	a
Phenanthrene	5000 (p)	LL-EAC	a
Pyrene	100 (p)	LL-EAC	a
sum7PCBs	5	LL-EAC	a
TBT	1	LL-EAC	a
PFOS	16.7	PNECoral	b

[1]a. OSPAR, 2004; b. OSPAR2006

The quality criteria for human health (Table VII and Table VIII) were either comprised of acceptable or tolerable daily intake values (ADIs or TDIs, respectively) expressed as µg contaminant uptake per kg body weight per day, or as maximum levels (ML) in µg of contaminant per kg fresh food. In case of ML values, the risk assessment is similar as for predators (i.e. the calculation of an RCR by dividing the concentration in food (the (P)EC) by the ML). In the case of an ADI/TDI value, however, data on food consumption are needed in order to calculate an RCR value. These data were retrieved from the EFSE Comprehensive European Food Database (EFSA, 2011). From this database, the food uptake for adults (expressed in g of food per kg body weight per day) was extracted for

water molluscs, crustaceans and fish meat. The mean uptake of these marine food sources for adults were 0.72, 0.41 and 0.80, respectively. The measured contaminant concentrations in mussels, shrimp and fish were converted to a fresh weight basis (if needed) in order to calculate the uptake of contaminants via the consumption of these organisms. This uptake was then expressed as μg contaminant per kg body weight per day, so an RCR could be calculated by dividing this uptake by the relevant ADI/TDI value.

Table VII. Quality criteria used for the assessment of human health risks of micropollutants. ADI: Acceptable daily intake; TDI: Tolerable daily intake. PAHs and PCBs are not included in this table.

Compound	ADI/TDI (μg/kg/d)	Ref.[1]
Chloridazon	100	a
Chlorpyriphos	10	a
Dichlorvos	0.08	a
Diuron	7	a
Isoproturon	15	a
Kepone	0.5	a
Metolachlor	80	a
Terbutylazine	3	a
Carbamazepine	0.34	b
Ofloxacin	3.2	c
Paracetamol	340	d
Propranolol	1.14	e
Salicylzuur	6.3	f
PFOA	1.5	g
PFOS	0.15	g
TBT	0.25	h
DBT	2.3	i
TPT	0.5	i

[1] a. EU, 2012; b. NACWA, 2010; c. FSCJ, 2007; d. Schwab, 2005; e. NRMMC, 2008; f. EU, 2010; g. EFSA, 2008; h. EFSA, 2004; i. RIVM, 2000

For human health as well as for secondary poisoning to predators, the risk assessment was also performed by using predicted concentrations in biota as obtained via equilibrium modeling with passive sampling data (§ 0). This novel approach is compared to the conventional methodology.

Table VIII. Quality criteria used for the assessment of human health risks of benzo(a)pyrene and PCBs. ML: maximum level; FT: fish tissue; BM: bivalve mollusc; Cr: crustaceans.

Compound	Value	Unit	QC	Reference
Benzo(a)pyrene	2	μg/kg WW	ML, FT	EU, 2006
	10	μg/kg WW	ML, BM	EU, 2006
	5	μg/kg WW	ML, Cr	EU, 2006
Sum(PCBs)	0.02	μg per kg BW per day	TDI	WHO,2003a

3. RESULTS

3.1. Exposure assessment: occurrence and geographical distribution of contaminants

3.1.1. Conventional analysis

All data of the conventional analysis are available on the restricted pages of the INRAM project website. What is described in the following paragraphs, are the most important results.

3.1.1.1. Organotin compounds

In sediment, concentrations of TBT up to 80 μg/kg dry weight were measured. Stations S22, OO1, ZB01 and ZB02 showed highest concentrations. Σ6organotins (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 Σ6organotins of water and sediment were put in a Box and Whisker plot (Figure 4).

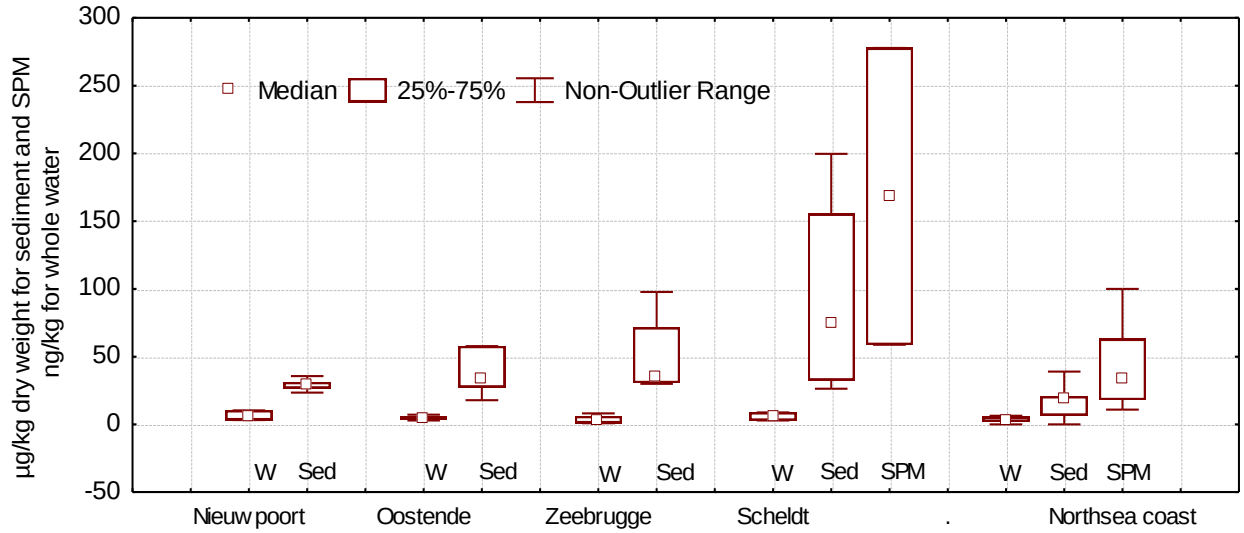

Figure 4. Box and Whisker plot for Σ6organotins grouped in 5 sampling regions, Nieuwpoort harbour, Oostende harbour, Zeebrugge harbour, the Scheldt Estuary and the Northsea coast in the water phase (W), Sediment (Sed) and Suspended matter (SPM).

3.1.1.2. PAHs

PAH concentrations measured in sediment were highest for the compound fluoranthene (up to 1000 μg/kg 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 Σ22PAHs 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 Σ22PAHs 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. Naphthalene, 2,6-dimethylnaphthalene, benzo(b)- and benzo(k)fluoranthene were the most abundant, with concentrations up to 75 ng L^{-1}. Concentrations of Σ22PAHs of water and sediment are to be viewed in a box and whisker plot (Figure 5).

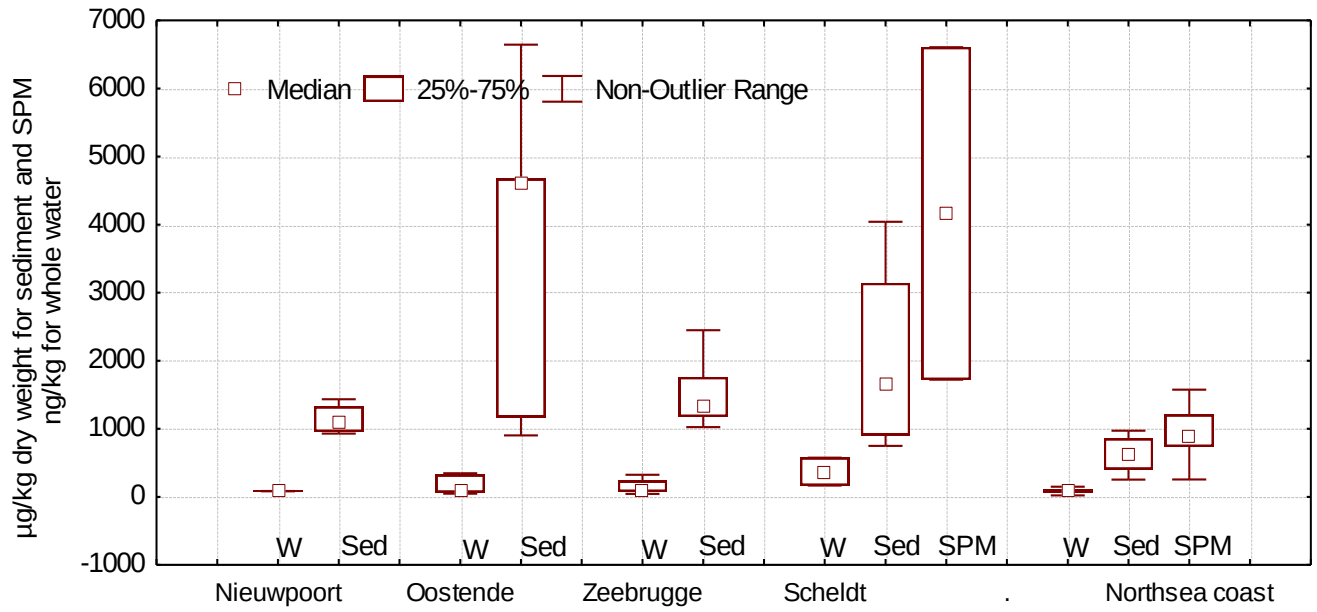

Figure 5. Box and Whisker plot for Σ_{22}PAHs grouped in 5 sampling regions, Nieuwpoort harbour, Oostende harbour, Zeebrugge harbour, the Scheldt Estuary and the Northsea coast in the water phase (W), Sediment (Sed) and Suspended matter (SPM).

3.1.1.3. 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. Σ6BDEs (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 Σ6BDEs varied from < 0.05 to 20 ng L-1 in the water phase. The Σ6BDEs 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 Σ6BDEs of water and sediment are presented in a Box and Whisker plot (Figure 6).

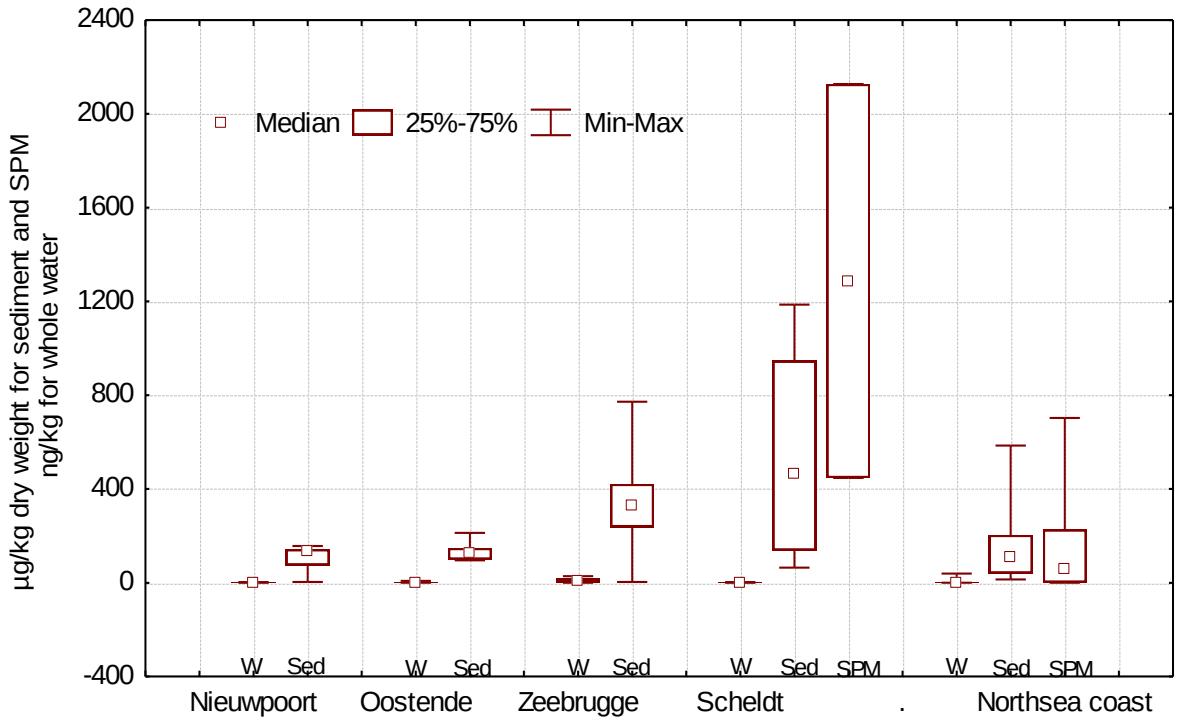

Figure 6. Box and Whisker plot for Σ6BDEs grouped in 5 sampling regions, Nieuwpoort harbour, Oostende harbour, Zeebrugge harbour, the Scheldt Estuary and the Northsea coast in the water phase (W), Sediment (Sed) and Suspended matter (SPM).

3.1.1.4. Phenols in water

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 $\mu g.L^{-1}$ was set for octylphenol and nonylphenol respectively. All concentrations of octylphenol were lower than the limit of detection (LOD) (= 0.015 $\mu g.L^{-1}$).

Figure 7 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 $\mu g.L^{-1}$. 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 $\mu g.L^{-1}$ 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.

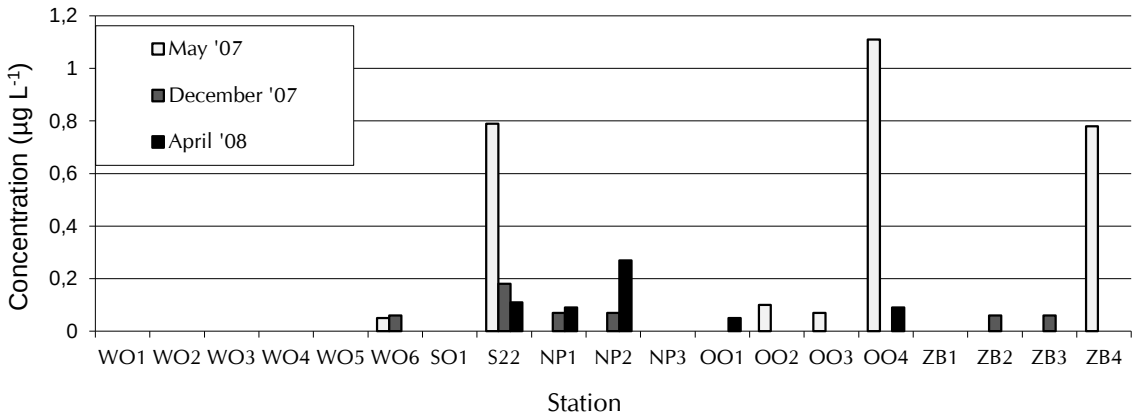

Figure 7. Concentrations of nonylphenol in the water column at the different sampling locations for the three sampling campaigns (LOD = 0.05 μg L-1).

3.1.1.5. Organonitrogen pesticides in water

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 IX). 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 IX.

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 $\mu g.L^{-1}$). Lower concentrations of isoproturon (< 0.05 $\mu g.L^{-1}$) were measured at the offshore sampling locations. Simazine was found in most harbour-samples. Measured concentrations were up to 0.060 $\mu g.L^{-1}$. For terbutylazine the highest concentrations (± 0.35 $\mu g.L^{-1}$) 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 IX. 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		

3.1.1.6. Pharmaceuticals in water

The newly developed method was applied to water samples collected during the INRAM project (see section of study area and sampling). Six offshore samples, 11 harbour samples and 2 samples of the Scheldt estuary were collected and analysed. This was repeated four times: in May and December 2007, April 2008 and June 2009. As can be deduced from Table X and Table XI, eight different pharmaceuticals were detected. The other pharmaceuticals were not detected at any of the sampling stations.

Table X. The detected concentrations of pharmaceuticals in water samples collected at the sampling stations at sea and on the Scheldt river. nd: not detected.

	Station							
Compound Sampling period	W01	W02	W03	W04	W05	W06	S01	S22
Salicylic acid								
May 2007	126	26	53	65	18	nd	51	372
Dec 2007	660	276	106	68	26	59	135	229
April 2008	102	56	62	88	65	49	91	71
June 2009	263	412	nd	227	237	60	307	264
Bezafibrate								
May 2007	nd	nd	nd	nd	nd	nd	nd	16
Dec 2007	nd	nd	nd	nd	nd	nd	nd	5
April 2008	8	nd	nd	nd	nd	nd	6	nd
June 2009	nd	nd	nd	nd	nd	nd	nd	nd
Sulfamethoxazole								
May 2007	nd	nd	nd	nd	nd	nd	nd	nd
Dec 2007	nd	nd	nd	nd	nd	nd	nd	30
April 2008	nd	nd	nd	nd	nd	nd	nd	96
June 2009	nd	nd	nd	nd	nd	nd	nd	nd
Trimethoprim								
May 2007	nd	nd	nd	nd	nd	nd	nd	nd
Dec 2007	nd	nd	nd	nd	nd	nd	nd	nd
April 2008	nd	nd	nd	nd	nd	nd	nd	nd
June 2009	nd	nd	nd	nd	nd	nd	nd	nd
Carbamazepine								
May 2007	18	nd	nd	7	nd	nd	5	321
Dec 2007	19	nd	nd	10	nd	nd	18	154
April 2008	16	14	4	12	nd	nd	27	185
June 2009	11	nd	nd	7	nd	nd	14	129
Propranolol								
May 2007	nd	nd	nd	nd	nd	nd	nd	22
Dec 2007	1	nd	nd	nd	nd	nd	1	10
April 2008	nd	nd	nd	nd	nd	nd	3	22
June 2009	nd	nd	nd	nd	nd	nd	nd	15
Atenolol								
May 2007	nd	nd	nd	nd	nd	nd	nd	89
Dec 2007	nd	nd	nd	nd	nd	nd	nd	293
April 2008	nd	nd	nd	nd	nd	nd	nd	188
June 2009	nd	nd	nd	nd	nd	nd	nd	nd

Sulfamethoxazole and trimethoprim were found up to concentrations of 96 and 29 ng L^{-1} respectively. Other antibiotics could not be detected. The widely used NSAID salicylic acid was detected very often. Salicylic acid, the deacylated more active form of acetylsalicylic acid, was detected in more than 90% of all samples at a concentration up to 855 ng L^{-1}. β-blocker propranolol was found in half of the samples in levels up to 24 ng L^{-1}, while atenolol was detected only six times up to 293 ng L^{-1}. Bezafibrate was detected at concentrations below 18 ng L^{-1} and residues of the psychiatric drug carbamazepine were frequently found in levels up to 321 ng L^{-1}. Salicylic acid and carbamazepine may be considered as the most relevant compounds for the North Sea and Scheldt Estuary since they were detected most often and at the highest concentrations.

Table XI. The detected concentrations of pharmaceuticals in water samples collected at the sampling stations in the coastal harbours. nd: not detected, nm: not measured.

	Station										
Compound Sampling period	OO1	OO2	OO3	OO4	NP1	NP2	NP3	ZB1	ZB2	ZB3	ZB4
Salicylic acid											
May 2007	246	855	nm	161	44	31	11	48	130	110	41
Dec 2007	598	365	234	104	99	94	121	136	142	271	197
April 2008	203	74	43	67	44	46	nm	114	87	80	146
June 2009	481	222	374	nd	306	nd	177	16	312	310	nd
Bezafibrate											
May 2007	5	18	nm	6	nd	nd	nd	nd	nd	nd	nd
Dec 2007	nd	7	12	11	nd	nd	nd	nd	nd	nd	nd
April 2008	5	9	7	6	nd	nd	nm	6	6	5	nd
June 2009	nd	18	10	nd	nd	nd	nd	nd	nd	nd	nd
Sulfamethoxazole											
May 2007	nd	nd	nm	nd	nd	nd	nd	nd	nd	nd	nd
Dec 2007	nd	nd	39	27	13	nd	nd	nd	nd	nd	nd
April 2008	nd	43	21	15	15	nd	nd	nd	nd	nd	nd
June 2009	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Trimethoprim											
May 2007	nd	15	nm	nd	nd	nd	nd	nd	nd	nd	nd
Dec 2007	nd	nd	nd	nd	nd	17	nd	nd	nd	nd	nd
April 2008	nd	29	nd	nd	nd	nd	nm	nd	nd	nd	nd
June 2009	nd	nd	13	nd	nd	nd	nd	nd	nd	nd	nd
Carbamazepine											
May 2007	31	119	nm	16	29	15	nd	11	10	11	11
Dec 2007	29	19	32	30	68	54	37	14	12	17	24
April 2008	30	64	35	36	48	20	nm	30	25	23	20
June 2009	21	50	36	20	19	7	nd	10	13	20	16
Propranolol											
May 2007	5	24	nm	3	6	3	nd	1	nd	nd	1
Dec 2007	3	6	9	9	12	12	7	1	1	4	3
April 2008	2	21	11	12	3	2	nm	3	3	2	2
June 2009	nd	13	3	nd	nd	nd	nd	nd	nd	nd	nd
Atenolol											
May 2007	nd	88	nm	nd	nd	nd	nd	nd	nd	nd	nd
Dec 2007	nd	nd	nd	80	nd	nd	nd	nd	nd	nd	nd
April 2008	nd	82	nd	nd	nd	nd	nm	nd	nd	nd	nd
June 2009	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Many studies have reported the presence of pharmaceuticals in rivers and in influents and effluents of WWTPs (Bendz et al., 2005; Lindqvist et al., 2005; Gros et al., 2009; Madureira et al., 2009). Observed concentrations depend on the therapeutic class. The presence of antibiotics in surface water is generally reported in the low ng L^{-1} concentration range, while several NSAIDs reach μg L^{-1} levels. The levels of salicylic acid measured in our study are within the same order of magnitude as those reported in literature for freshwater samples. Detected concentrations of β-blockers and lipid regulators in the Belgian coastal zone are rather low in comparison to concentrations detected in surface water (Bendz et al., 2005; Gros et al., 2006). On the other hand, carbamazepine occurs at high levels and is very often detected. This can be explained by its low removal efficiency in WWTPs (Grujic et al., 2009; Ternes 1998). With regard to marine waters, Buser et al. (1998) and Weigel et al. (2001 and 2002) reported the

presence of clofibric acid and carbamazepine in the North Sea at concentrations of approximately 1 and 2 ng L^{-1}, respectively. More recent, Togola and Budzinski (2008) reported higher concentration levels for carbamazepine, diclofenac and ketoprofen in the Mediterranean Sea. However, in general, monitoring data for pharmaceuticals in the marine environment are rather sparse. This may reflect the absence of a method to extract and identify multi-class pharmaceuticals in marine systems (Weigel et al., 2001). In this study, a novel multi-class analytical method for pharmaceuticals was developed clearly showing the ubiquitous character of several selected pharmaceutical compounds in seawater and as a result in the marine environment as well. These findings will assist to further research within this project, in which risk assessments of the detected pharmaceuticals for the marine environment will be performed.

The concentrations of some pharmaceuticals under investigation show large temporal variations. However, except for salicylic acid at W01 and W02, the same general picture of lower concentrations in the North Sea (W01-W06) in comparison with the harbours and the Scheldt estuary could be noticed. It can be suggested that, due to both dilution and degradation, there is little transport from the Scheldt estuary and the harbours to the open sea. Also previous studies about estrogens, nonyl phenol ethoxylates (NPEs), and polychlorinated biphenyls (PCBs), reported the limited transport from the Scheldt estuary to the North Sea (Vuksanovic et al., 1996; Jonkers et al., 2005; Noppe et al., 2006). At sampling locations OO2 and S22, targeted pharmaceuticals were detected most frequently and at the highest concentrations. OO2 is situated at the mouth of the river Noord-Ede and the canal Bruges-Ostend in the harbour of Ostend (in the middle of the Belgian coastal zone), while S22 is located at the Scheldt estuary in Antwerp. Several WWTPs are located close to OO2 and in Flanders more than 65 WWTPs effluents are discharged into the Scheldt. Furthermore, WWTPs with the largest capacity are located in the vicinity of the Scheldt estuary: Deurne, Ghent and Antwerp (approximately 200,000 Inhabitant Equivalent) [Aquafin, personal communication]. It may be concluded that both locations receive major inputs of contaminated industrial and domestic wastewater, probably resulting in the increased presence of the targeted pharmaceuticals.

3.1.1.7. Perfluorinated compounds in water

The developed method was applied to water samples collected during the INRAM project (see section 2.1). Six offshore samples, 11 harbour samples and 2 samples of the Scheldt estuary were collected in June 2009. As can be seen from Table , four different

PFCs were detected in all waters samples. The other PFCs were not detected at any of the sampling stations. PFOS was detected in every sample in levels up to 38.9 ng L^{-1}, while PFOSA was found once at a concentration of 26.4 ng L^{-1} at sampling location S22 in Antwerp. PFHxS and PFOA were frequently detected up to concentrations of 13.1 and 23.5 ng L^{-1} respectively, both at sampling location S22 (Antwerp).

Despite the limited monitoring study, certain differences could be observed between the sampling stations in the study area. As can be seen from Table XII, the harbour of Ostend and in particular the Scheldt estuary were most contaminated with PFCs. Analysis of the S22-sample resulted in the detection of 4 different PFCs, up to 38.9 ng L^{-1}. Since S22 is located in the industrial zone of Antwerp, large inputs of PFCs could be expected. Samples of the North Sea (W01-W06) were the least contaminated with PFCs. At these locations, only PFOS could be quantified in concentrations below 5 ng L^{-1}. These concentrations of PFOS were in the same range as those reported in previous studies of the German Bight, which is the southeastern bight of the North Sea (Ahrens et al. 2009; Caliebe et al., 2004; Theobald et al., 2007). In accordance with Ahrens et al. (2009), a decreasing contamination of PFCs with increasing distance from the coast could be observed.

Table XII. The detected concentrations of perfluorinated compounds in water samples. nd: not detected.

	W01	W02	W03	W04	W05	W06	S01	S22	NP1	NP2	NP3	OO1	OO2	OO3	OO4	ZB1	ZB2	ZB3	ZB4
PFPA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFBS	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFHxA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFHpA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFHxS	d	nd	nd	d	nd	nd	3.7	13.1	nd	nd	nd	d	6.7	2.9	2.4	d	d	d	d
PFOA	nd	nd	nd	nd	nd	nd	d	23.5	nd	nd	nd	nd	d	nd	nd	nd	nd	nd	nd
PFNA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFOS	4.2	2	d	2.9	d	d	5.2	38.9	3.6	2.9	2.3	6.5	12.8	5.9	4.3	4.8	4.7	3.3	4.4
PFDA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFDS	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFUnA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFDoA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFOSA	nd	nd	nd	nd	nd	nd	nd	26.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFTeA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Compared to the derived PNEC values of 25 μg L^{-1} for PFOS and 250 μg L^{-1} or 1.25 mg L^{-1} for PFOA (E. De Wulf, Flemish Environment Agency (FEA), personal communication,

and Colombo et al., 2008) adverse risks to aquatic organisms are not anticipated from these measured concentrations. However, the possible combined effects that the abundance of several PFCs may cause, possibly even with other micropollutants, cannot be excluded. Next to these toxicity thresholds, two studies determined a health-based guidance for PFOA in drinking water as well. According to the US Safe Drinking Water Act (2004), these studies reported drinking water equivalent levels (DWEL: the lifetime exposure level of a contaminant at which adverse health effects are not anticipated to occur, assuming 100% exposure from drinking water). Tardiff et al. (2009) found DWELs for PFOA ranging from 0.88 to 2.4 μg L^{-1}, while a guidance value of 0.040 μg L^{-1} was recommended by Post (2009). The detected PFOA-concentrations did also not exceed these drinking water levels.

3.1.1.8. Analysis of pharmaceuticals, pesticides and perfluorinated compounds in biota

The developed methods were applied to *M. edulis* samples, derived from two cage experiments (see Section 2.1). As shown in Table XIIITable XIV, five different pharmaceuticals were detected in the *M. edulis* samples. The widely used non-steroidal anti-inflammatory drug (NSAID) salicylic acid was found in almost every sample in levels up to 490 ng g^{-1}. A second NSAID, namely paracetamol was detected less frequently in concentrations up to 115 ng g^{-1}. Also the β-blocker propranolol and the antibiotic ofloxacin were measured in some samples: up to 63 and 65 ng g^{-1}, respectively. Finally, carbamazepine was detected in concentrations ≤ 11 ng g^{-1}. Salicylic acid has been identified by Wille et al. (2010) as the most prevalent pharmaceutical in water samples collected in the Belgian coastal waters, which explains the presence of this compound in the *M. edulis* samples observed in this study. No obvious temporal trends could, however, be observed during the cage experiments. Moreover, the measured concentrations of salicylic acid showed large variations over time and location.

Seven target pesticides were found in the *M. edulis* samples originating from the cage experiments. The concentrations of 5 pesticides (diuron, linuron, isoproturon, metolachlor, terbutylazine) were close to the limit of quantification, while the detected concentrations of chloridazon and dichlorvos were significantly higher. Chloridazon was observed up to 16 ng g^{-1} and dichlorvos was found in most samples with a maximum of 60 ng g^{-1}. This implies that the European default maximum pesticide residue level (MRL) in foodstuffs of 10 ng g^{-1} (EC 396/2005), was exceeded for

chloridazon and dichlorvos at several stations. Carafa et al. (2007) also reported the exceeding of this MRL in clams, in which up to 73 ng g^{-1} of terbutylazine was retrieved.

Table XIII. Detected concentrations (ng g^{-1}) of the target micropollutants in *Mytilus edulis* deployed in a 6-month cage experiment performed at five stations in the Belgian coastal zone (nd = not detected; na = not analyzed).

	Sampling location																		
	ES	ZB1					ZB2					Sea				NP		OO	
	Feb	Mar	Apr	May	Jun	Jul	Mar	Apr	May	Jun	Jul	Apr	May	Jun	Jul	Mar	Apr	Mar	Apr
Pharmaceuticals																			
Ofloxacin	nd	nd	nd	nd	nd	5	nd	5	nd	nd	nd	nd	8	nd	7	nd	nd	nd	65
Propranolol	nd	40	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	19	nd	nd	52
Salicylic acid	145	444	208	223	nd	172	162	241	339	184	315	264	93	203	119	490	125	41	206
Carbamazepine	1	11	3	1	1	3	nd	4	nd	3	nd	nd	nd	2	nd	nd	nd	3	nd
Paracetamol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	96	nd	nd	nd	nd	nd	115	65
PFCs																			
PFHxS	nd	nd	nd	nd	nd	3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFOS	1	4	4	4	3	4	2	4	3	1	1	1	nd	nd	nd	nd	nd	2	3
Pesticides																			
Dichlorvos	21	18	19	5	25	23	20	15	10	19	5	4	nd	20	28	5	11	na	14
Diuron	1	1	1	nd	1	1	1	1	1	1	1	nd	nd	nd	nd	nd	nd	na	1
Linuron	nd	2	nd	nd	1	nd	nd	nd	nd	1	nd	nd	nd	nd	nd	1	nd	na	nd
Cloridazon	nd	nd	nd	nd	16	nd	nd	nd	nd	nd	nd	nd	nd	nd	11	nd	nd	na	nd
Isoproturon	nd	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	n.d	nd	na	nd
Metolachlor	nd	nd	nd	nd	nd	1	nd	1	nd	1	1	nd	nd	nd	nd	1	1	na	1
Terbutylazine	nd	nd	nd	nd	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1	nd	na	nd

Table XIV. Detected concentrations (ng g^{-1}) of the target micropollutants in *Mytilus edulis* deployed in a 6-week cage experiment performed at four stations in the Belgian coastal zone (nd = not detected).

	Sampling location				
	ES	ZB1	ZB2	NP	OO
Pharmaceuticals					
Ofloxacin	nd	nd	nd	nd	nd
Propranolol	nd	39	38	30	63
Salicylic acid	33	14	118	288	229
Carbamazepine	nd	3	1	4	4
Paracetamol	nd	nd	nd	nd	115
PFCs					
PFHxS	nd	nd	nd	nd	nd
PFOS	nd	5	2	nd	nd
Pesticides					
Dichlorvos	25	7	8	60	8
Diuron	nd	1	1	nd	nd
Linuron	nd	nd	nd	nd	nd
Cloridazon	13	8	7	6	nd
Isoproturon	nd	nd	nd	1	1
Metolachlor	1	nd	nd	nd	1
Terbutylazine	nd	nd	nd	1	1

Only two PFCs were detected in the *M. edulis samples*; perfluorohexane sulfonate (PFHxS) was detected only once at a concentration of 3 ng g^{-1}, while perfluorooctane sulfonate (PFOS) was found in most samples at levels ≤ 5 ng g^{-1}. These concentrations

were in the same order as those reported by So et al. (2006) who found PFHxS and PFOS in mussel samples at levels ≤ 4 ng g^{-1} in coastal waters of China and Japan. In the study of Van de Vijver et al. (2003)], much higher concentrations of PFOS were measured in aquatic invertebrates of the southern North Sea, which is the same study area as the present study. PFOS was measured in shrimp (*Crangon crangon*), crab (*Carcinus maenas*), and starfish (*Asterias rubens*) up to 520 ng g^{-1}, 877 ng g^{-1}, and 176 ng g^{-1}, respectively.

3.1.1.9. Pb and Cd in marine sediments

Table XV presents results of the analysis of Pb and Cd in sediments. A first examination of the results learned that in 2008, the concentrations of both metals in sediments were the highest at station S22 (Antwerp). The concentrations observed in the harbours were elevated compared to stations at sea. However, in 2009 the concentrations observed at station S22 were much lower and no longer the highest. This can be partly explained by the granulometry of the sediment, which reveiled that in 2009 the sediment collected at S22 exhibited no fraction smaller than 63 μm, compared to 46.2 % in 2008. Further statistical analysis including the sediment granulometry and TOC content of the sediments will be necessary to investigate and interpret the observed patterns of metal contamination along the Belgian coastal zone.

Table XV. Concentrations of lead (Pb) and cadmium (Cd) in sediments collected during the sampling campaigns of 2008 and 2009. Standard deviation is given between brackets. The last two columns indicate the sediment fraction smaller than 63 μm (i.e. the clay fraction) in the respective sediment samples. DW: Dry Weight.

	Pb concentration (μg/g DW)		Cd concentration (μg/g DW)		Sediment ≤ 63μm (%)[1]	
Station	2008	2009	2008	2009	2008	2009
OO1	37.9 (3.0)	60.7 (1.6)	0.90 (0.03)	1.02 (0.05)	80.4	84.9
OO2	28.1 (3.1)	43.4 (0.6)	0.62 (0.02)	0.54 (0.05)	87.5	91.6
OO3	28.8 (0.4)	40.1 (2.7)	0.64 (0.22)	0.54 (0.04)	89.2	93.5
OO4	17.5 (1.2)	33.0 (1.0)	0.84 (1.13)	0.40 (0.01)	65.5	83.5
NP1	27.4 (0.3)	36.0 (1.8)	0.59 (0.14)	0.52 (0.03)	86.7	90.0
NP2	14.8 (0.3)	31.3 (2.1)	0.32 (0.00)	0.46 (0.01)	90.9	85.6
NP3	10.7 (0.4)	7.8 (0.1)	0.20 (0.01)	0.15 (0.01)	31.1	26.4
ZB1	33.2 (0.8)	46.0 (0.9)	0.55 (0.02)	0.70 (0.13)	91.7	84.5
ZB2	31.5 (0.5)	43.3 (2.0)	0.54 (0.06)	0.56 (0.10)	91.5	95.8

ZB3	34.1 (0.5)	43.9 (0.6)	0.70 (0.01)	0.59 (0.12)	91.0	84.8
ZB4	25.8 (0.4)	31.9 (0.6)	0.43 (0.03)	0.39 (0.01)	84.4	78.7
W01	14 (0.5)	8.5 (0.3)	0.24 (0.01)	0.12 (0.02)	57.6	83.5
W02	12.1 (0.1)	6.7 (0.2)	0.22 (0.02)	< DL	56.0	83.0
W03	4.7 (0.2)	6.8 (0.3)	< DL	< DL	0.0	11.0
W04	4.4 (1.0)	10.1 (0.1)	< DL	< DL	0.0	63.6
W05	3.7 (1.3)	6.9 (0.2)	< DL	< DL	1.1	1.3
W06	1.8 (0.2)	5.1 (0.6)	< DL	< DL	0.0	0.0
S01	14.7 (0.7)	8.0 (0.4)	0.19 (0.01)	< DL	47.8	0.0
S22	216.8 (15.3)	22.2 (2.2)	8.52 (0.18)	0.20 (0.02)	46.2	0.0

[1]Bold values indicate a difference of more than 25% between the subsequent sampling years

3.1.2. Passive sampling: determination of freely dissolved, time-weighted average concentrations

An accurate and reliable method of measuring concentrations of organic marine contaminants remains a major challenge in controlling pollution in the sea, due to the low concentrations of POPs and due to the uncertainty of the bioavailability of these POPs. Passive samplers can be the answer to above-mentioned problems. Detection limits can be sensitively lowered with the use of this technique (up till 0.001 ng L^{-1}). Also passive sampling estimates the concentration of POPs freely dissolved in the water phase, i.e. the bio-available fraction and not the concentration bound to suspended particles and humic acids, also known as total water concentration. POPs are mostly very hydrophobic and bind easily to sediment, which often acts as a sink for POPs. Resuspension of POPs is possible due to disturbing the sediment layer by e.g. dredging. A comparison between concentrations from grab sampling and concentrations from passive sampling will be made during this project. In Figure 8 results are shown comparing passive sampling and grab sampling of PAH concentrations.

Concentrations of PAHs in grab samples of total water were measured 3 times during 2007. The average concentration of the sum of the 12 lower MW PAHs was compared to the concentrations of these PAHs in passive samplers from 2007 (Figure 8). A passive sampler estimates the concentration of PAHs freely dissolved in the water phase and not the concentration bound to suspended particles and humic acids, also known as total water concentration. Variation of concentrations between different stations of more then factor 10 was seen in passive samplers. As this concentration is integrative over time, this could indicate actual lower pollution of dissolved PAHs on these sites.

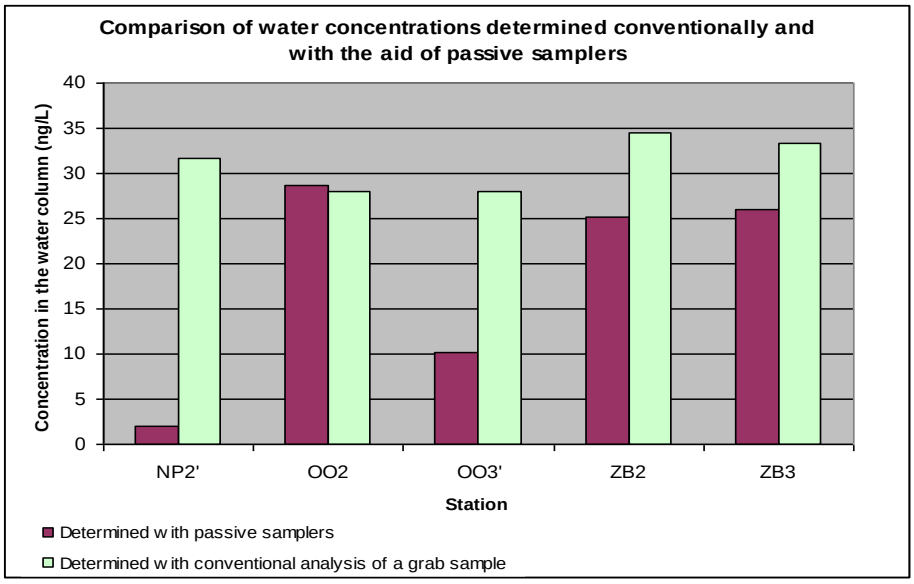

Figure 8. Concentrations of sum12PAHs measured in passive samplers from 2007 (purple coloured) and average total water concentrations (n = 3) of sum12PAHs measured 3 times during 2007 (green coloured) in ng L^{-1}.

Generally Nieuwpoort harbour showed lowest contamination with PAHs. This also corroborates with the measurements in total water and sediment. Oostende and Zeebrugge showed highest concentrations, with sum22PAHs measured in passive samplers varying from 14.02 to 118.96 ng L^{-1}. The contaminants fluorene, 2,6-dimethylnaphthalene and acenaphthene were most abundant. This indicates contamination of petrogenic origin, possibly from diesel-fueled vessels.

A passive sampler only measures the concentration of POPs freely dissolved in the water phase, integrated over the period of deployment. This concentration is up till now unknown for the Belgian coastal zone. Figure 9 shows dissolved concentrations of sum22PAHs measured with the passive samplers in the water.

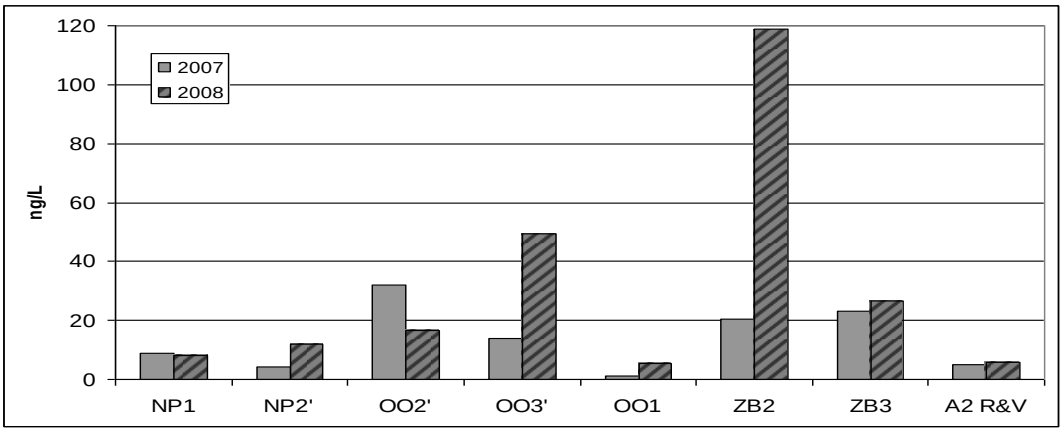

Figure 9. Dissolved concentrations of Sum22PAHs in the water phase expressed in ng L-1 measured with passive samplers during 2007 (grey coloured) and 2008 (striped).

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}). Passive samplers measure the truly dissolved fraction of contaminants, thus lower concentrations of higher molecular weight (MW) PAHs is probably due to the lower water solubility. The trend of lower concentrations of lower MW PAHs was not seen in total water measurements of the PAHs in 2007 and 2008 and indicates a difference between both active sampling and passive sampling. Total water grab sampling includes suspended solids and these particle bound PAHs could partly explain this difference.

In Figure 10 concentrations are shown of sum14PCBs in passive samplers deployed in 2007 and 2008. Concentrations were calculated to the water phase concentrations (ng L^{-1}). Following PRCs were used: CB10, CB50, CB104, CB145 and CB204 were used. However, due to low recovery of CB10, the PRC could not be used in the calculations for Rs. Further investigation showed that spiking concentrations of CB10 were too low for the GC-MS-MS detection. Higher spiking concentrations of PRCs were used in future experiments.

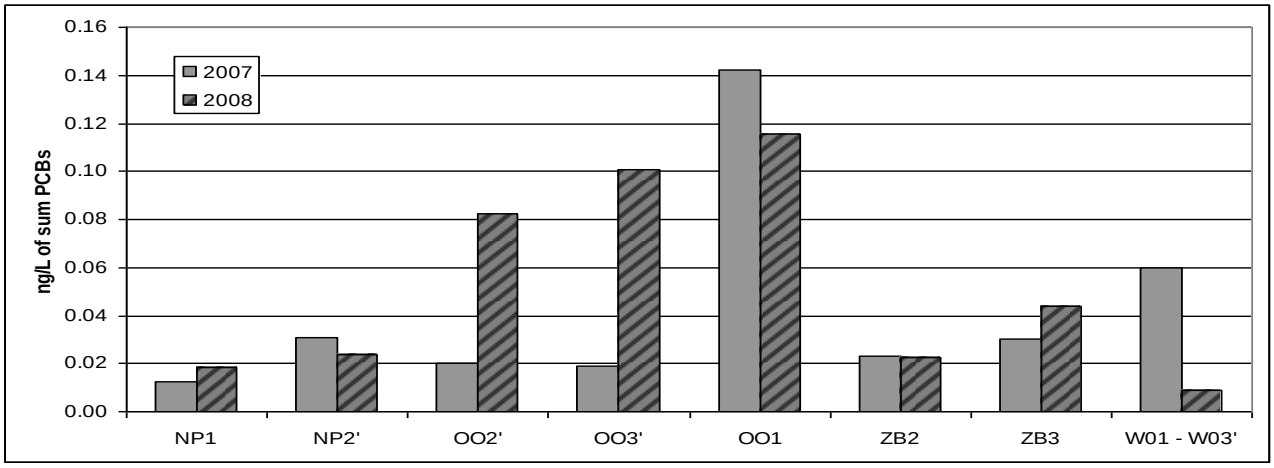

Figure 10. Dissolved concentrations of Sum14PCBs in the water phase expressed in ng L^{-1} measured with passive samplers during 2007 (grey coloured) and 2008 (striped).

The measured concentrations were very low. Dissolved concentrations in water for individual PCBs in 2007 varied from 0.001 ng L^{-1} to 0.04 ng L^{-1}, while for individual PAHs the concentrations varied from 0.001 ng L^{-1} to 70 ng L^{-1}. The sum14PCBs calculated to the water phase varied from 0.01 to 0.14 ng L^{-1}. Highest freely dissolved concentrations of PCBs were found in OO1 in 2007 as well as in 2008.

Concentrations of contaminants measured by passive samplers allows the estimation of the bio-available fraction and, what could be termed as pollution pressure on the aquatic eco-system. An international scale, representative for this pressure, would enable us to express contaminant levels in a framework of integrated environmental ecosystem monitoring. In order to do this, a link to ecotoxicity should be made. Figure 11 illustrates, in a simplified way, how contaminant pressure scale could be interpreted.

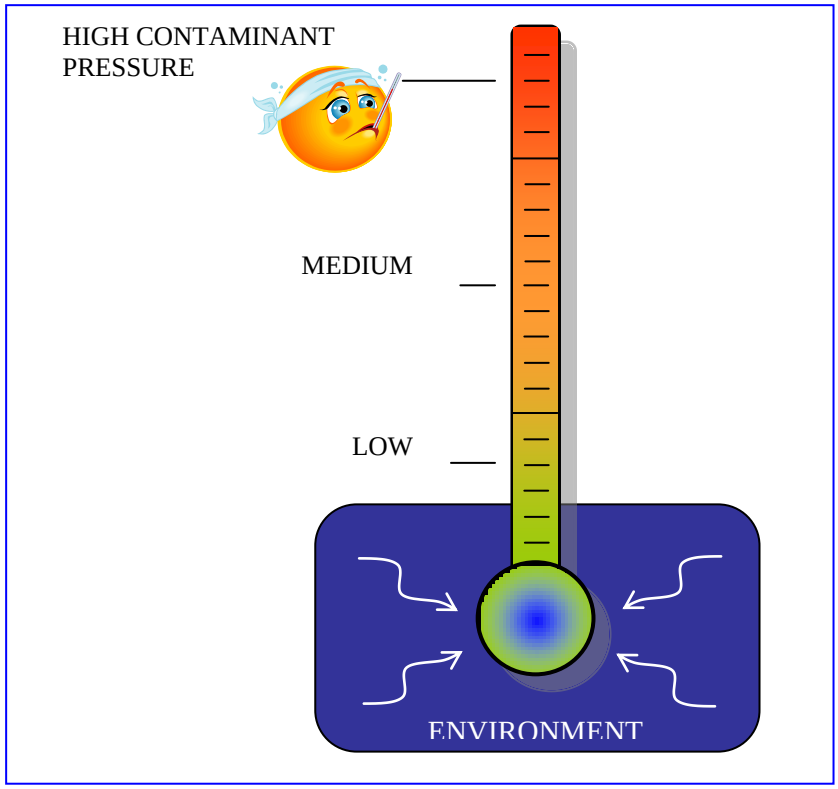

Figure 11. Measuring the temperature of the marine aquatic environment by measuring contaminant pressure.

This proposed international scale, bearing sufficient normalisation in mind, would essentially enable us, via passive samplers, to interpret the measured concentration directly and hence, the pressures on the aquatic eco-system. Current work related to OSPAR Environmental Assessment Criteria (EAC) and to the environmental quality standards (EQS) of the Water Framework Directive could benefit from this interpretation of contaminant pressure. This would constitute a major step in environmental status assessment. Concentrations measured in this project will be used to study the applicability of environmental criteria to passive sampling data.

The passive samplers were not analysed for the more polar target analytes, being the phthalates, perfluorinated compounds and phenols. For the phthalates, perfluorinated compounds and phenols, using these passive sampling devices, the risk for contamination was too high. Therefore, the passive samplers were not analysed for these three groups of compounds. On the other hand, new extraction procedures and analytical methods were developed for the quantification of a high number of pharmaceuticals and pesticides in the polydimethylsiloxane (PDMS) passive sampling devices (Wille et al., 2012).

3.1.3. Passive samplers as a central tool to monitor the distribution of pollutants

3.1.3.1 Sediment and SPM

The equilibrium model performed poorly for sediment and SPM, which was illustrated by the model bias (MB) indicating a general underestimation of the concentrations in sediment and SPM by a factor of 50 (see Table XVI). Figure 12A and B confirmed this bias but nonetheless indicated a relatively strong relationship between observed and predicted data. A regression analysis of the observed versus the predicted data yielded R^2 values of 0.43 and 0.53 and slopes of 0.86 and 0.70 for sediment and SPM, respectively. A potential explanation is the tendency of literature K_{oc} values to be an underestimate of field K_{oc} values. This was for example observed by Hawthorne et al. (2006), who found that field K_{oc} values typically ranged up to two orders of magnitude higher than literature values. In an attempt to improve the performance of the equilibrium model for our sediment and SPM data, we calculated $\log K_{oc,field}$ values from our data as follows:

$$\log K_{oc,field} = \log \frac{C_{sol} \cdot f_{oc}}{C_{diss}} \quad (8)$$

Table XVI. Percentage of modelled PAH and PCB concentrations in sediment and SPM that fall within a specific factor (as given in the first column) of the measured data. Results are given for both uncorrected and corrected models. The last row represents the model bias. N: number of data points; NC: not corrected; C: corrected; MB: model bias

	Sediment (N=322)		SPM (N=48)	
	NC	C	NC	C
Factor 2	0.9	38.8	0	43.8
Factor 5	3.7	79.8	2.1	66.7
Factor 10	9	89.6	12.5	89.6
Factor 100	67.1	99.1	68.8	100
Factor 1000	96.3	100	96.3	100
MB	0.02	1.01	0.02	0.98

The difference between $\log K_{oc,field}$ and $\log K_{oc}$ was on average 1.76 for sediment and 1.78 for SPM. Following this, we calculated corrected $\log K_{oc}$ values ($\log K_{oc,corr}$) as follows:

$$\log K_{oc,corr} = \log K_{oc} + 1.77 \quad (9)$$

Using $K_{oc,corr}$, the model performance increased significantly (Figure 12C and D) as the MB was very close to 1 for both sediment (1.01) as SPM (0.98), roughly 90% of the predicted values was accurate within an order of magnitude and approximately 40% was accurate within a factor of 2 for both matrices (Table). While the accuracy of $logK_{oc,field}$ should be further evaluated in future studies, the performance of the model is at this point remarkable given that the predicted data were compared to measured data from limited grab samples. Should more data be used for the comparison, the model performance is likely to further improve.

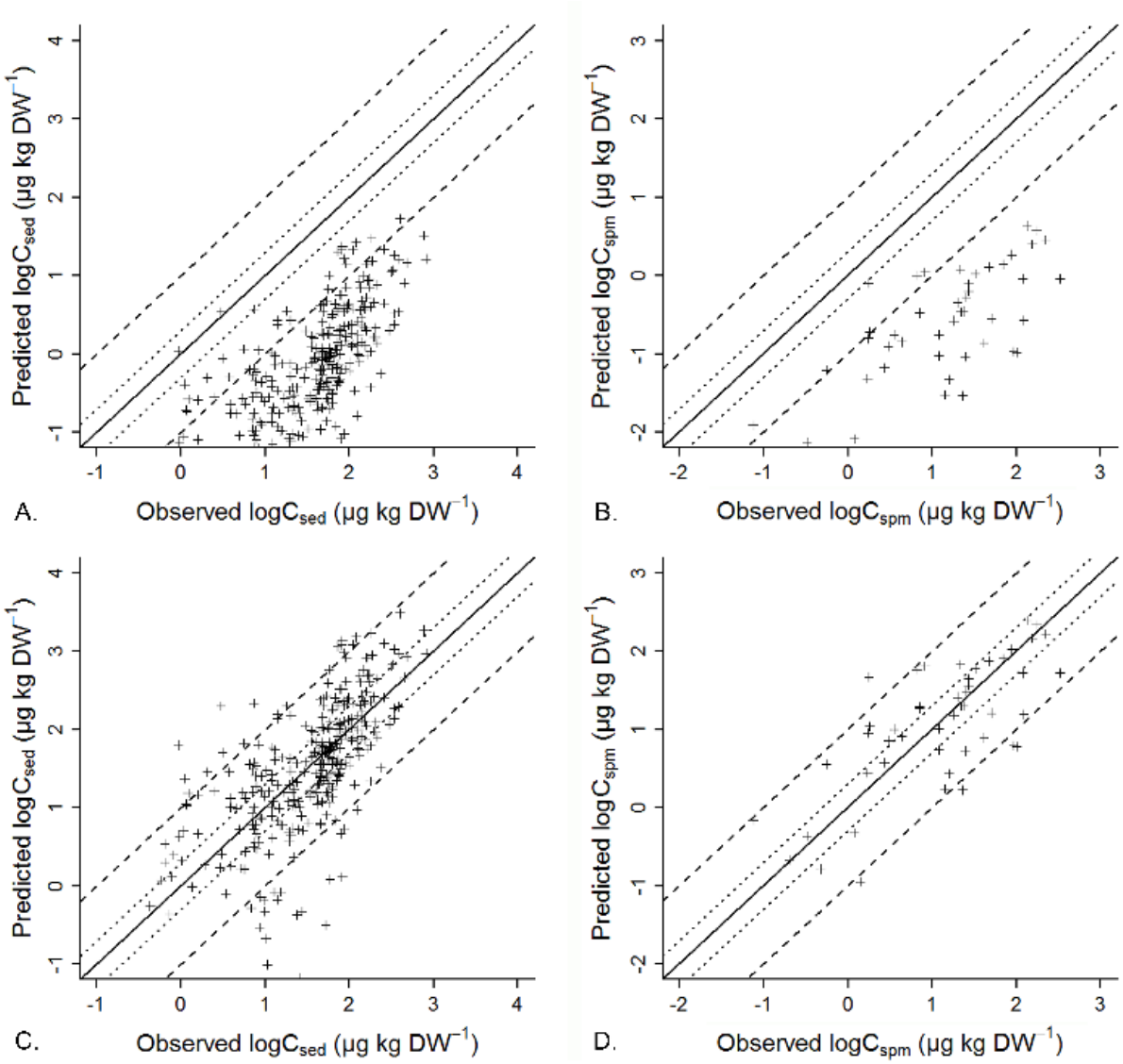

Figure 12. Predicted vs. observed concentrations of PAHs and PCBs in sediment and SPM. The solid line represents the 1:1 relationships, dotted and dashed lines represent a deviation of a factor of 2 and 10, respectively. A. Sediment, uncorrected; B. SPM, uncorrected; C. Sediment, corrected; D. Sediment, corrected.

3.1.3.2. Biota

The performance of the equilibrium approach was better for biota than it initially was for sediment and SPM. Between 68% (fish liver) and 90% (fish muscle tissue) of the predicted data was accurate within an order of magnitude of the measured values, and between 47% (fish liver) and 73.3% (shrimp) were accurate within a factor of 5 (Table). The MB did indicate a tendency of the model to overestimate the data with a factor up to 3.65. Further investigation of the data showed that this bias was mainly caused by compounds with a $\log K_{ow}$ above 6.5, and that this bias got stronger as $\log K_{ow}$ further increased (Figure 13). This was unexpected, as it is generally acknowledged that assimilation of contaminants via uptake of contaminated food becomes an increasingly important contributor to the body burden with increasing hydrophobicity. Thus, an equilibrium model would rather underestimate data as $\log K_{ow}$ increases. Possibly, the loss of a linear relationship between logKow and uptake of contaminants in organisms, which has been described in literature extensively (e.g. 32–36) could be the explanation. While there is no scientific consensus on the cause, this phenomenon has for example been attributed to lower bioavailability and a higher significance of the elimination via feces of very hydrophobic compounds (36, 37). Other scientists claim it to be an artifact arising from so-called third phase effects and nonequilibrium conditions occurring during laboratory bioconcentration measurements (38). While the real reason for the deviation observed in this study cannot be deducted from our data, a few comments can be made. As the concentration data deducted from passive sampling represent the freely dissolved and thus per definition represent the bioavailable fractions of the contaminants, a reduced bioavailability cannot be the reason for the observed discrepancy. What remains is the possibility of a lower uptake rate by organisms combined with growth dilution and/or a higher excretion rate of the highly hydrophobic contaminants, thereby causing a diversion from equilibrium conditions. In an attempt to perform a correction on the data, the relationship between $\log K_{ow}$ and the logarithm of the ratio of observed and predicted data (Figure 13) was determined for compounds with a $\log K_{ow} > 6.5$ (R^2 =0.34, N=103):

$$\log\left(\frac{C_{biota,observed}}{C_{biota,predicted}}\right) = 5.31 \cdot \log K_{ow} - 0.79 \qquad (10)$$

The resulting regression parameters were used to correct the predicted values of compounds, effectively transforming the slope in Equation 10 to zero. After this correction, between 76% (fish liver) and 100% (shrimp) of the predicted data was accurate within an order of magnitude of the measured values, and between 65% (fish liver) and 90% (fish muscle tissue) were accurate within a factor of 5 (Table XVII). Moreover, the MB values were

very close to 1 for all organisms. This is a significant improvement compared to the performance of the uncorrected model, however the general applicability of this corrected model should be verified in future research.

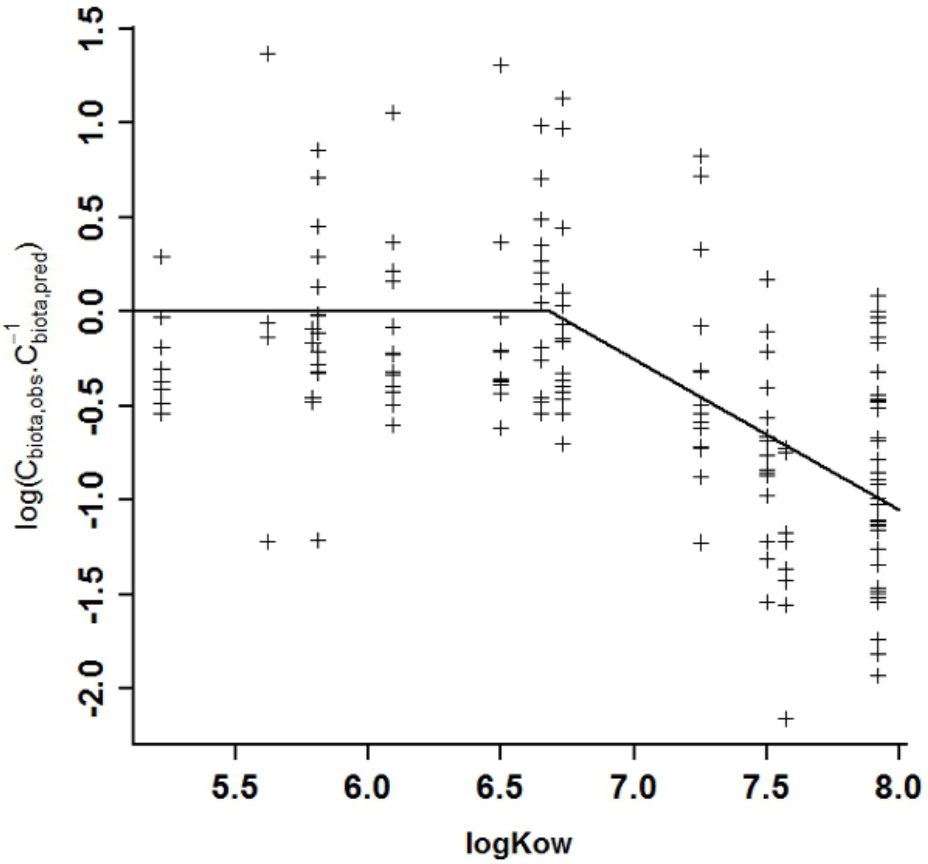

Figure 13. The logarithm of the ratio of observed and predicted concentration data in biota (pooled data from shrimp, mussels, flatfish liver and flatfish tissue) vs. logKow

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Table XVII. Percentage of modelled PAH and PCB concentrations in shrimp, mussel tissue, fish liver and fish muscle that fall within a specific factor (as given in the first column) of the measured data. Results are given for both uncorrected and corrected models. The last row represents the model bias. N: number of data points; NC: not corrected; C: corrected; MB: model bias.

	Shrimp (N=15)		MT (N=101)		FL (N=34)		FT (N=10)	
	NC	C	NC	C	NC	C	NC	C
Factor 2	20	40	30.7	46.5	26.5	32.4	30	60
Factor 5	73.3	73.3	68.3	88.1	47.1	64.7	70	90
Factor 10	73.3	100	82.2	97	67.6	76.5	90	90
Factor 100	100	100	100	100	97.1	100	100	100
MB	3.65	0.98	2.66	1.2	2.08	0.95	1.3	0.97

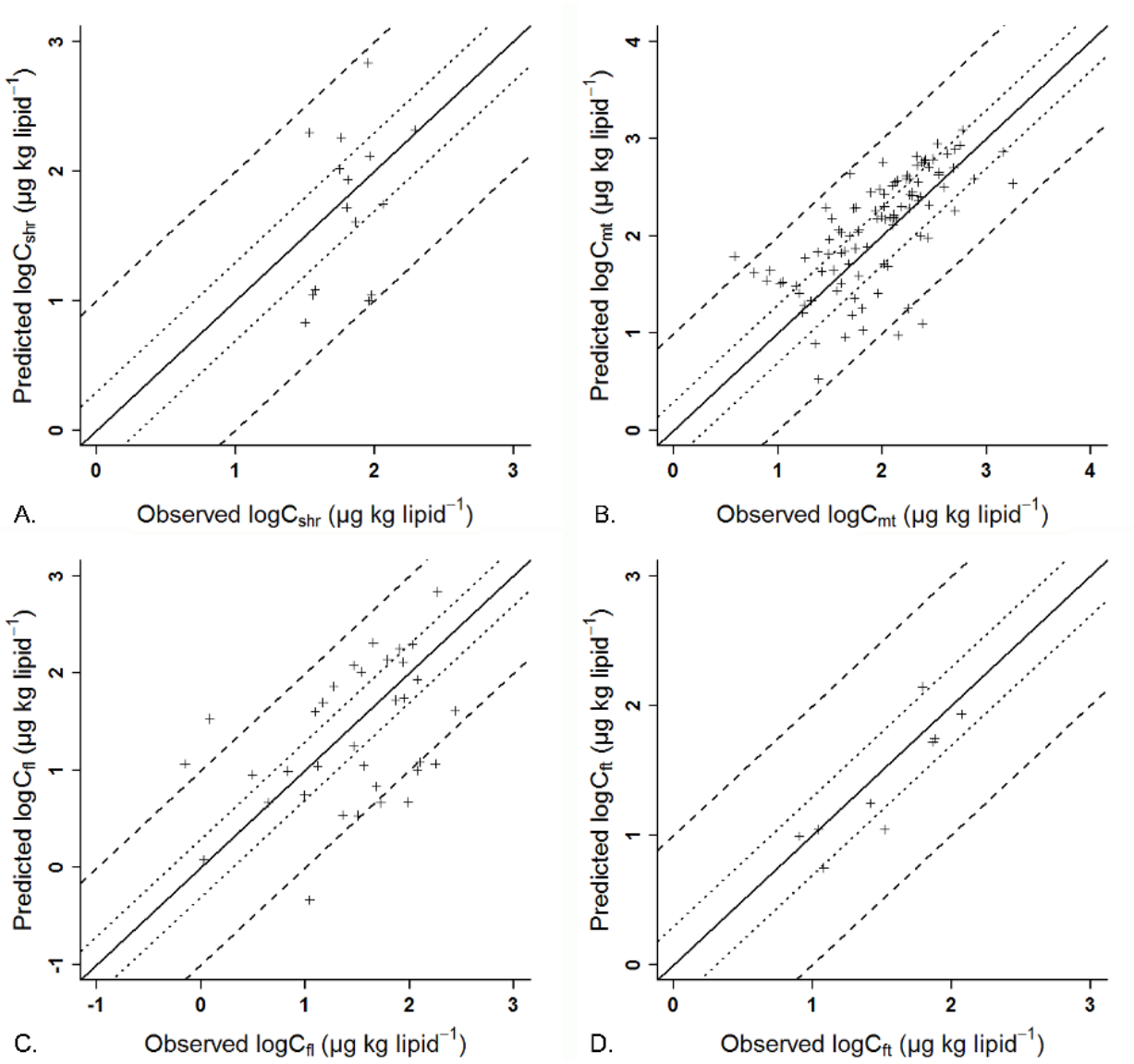

Figure 14. Predicted vs. observed concentrations of PAHs and PCBs in biota using the model with data correction for compounds with logKow > 6.5. The solid line represents the 1:1 relationships, dotted and dashed lines represent a deviation of a factor of 2 and 10, respectively. A. Shrimp; B. Mussels; C. Fish liver; D. Fish tissue.

3.1.4. Effects assessment

3.1.4.1. Single toxicant tests

The results of single toxicant tests performed with *P. tricornutum* (72h growth inhibition test) and *C. gigas* (24h larval development test) are presented in Table XVIII.

Table XVIII. Results of the *P. tricornutum* 72h growth inhibition tests and the *C. gigas* 24h larval development test for single substances (pharmaceuticals).

Compound	*P. tricornutum* EC50	EC10	C. gigas EC50 range*
Salicylic acid	255.5 mg L^{-1}	96.7 mg L^{-1}	> 100 mg L^{-1}
Carbamazepine	62.5 mg L^{-1}	42.2 mg L^{-1}	10 – 100 mg L^{-1}
Atenolol	877.1 mg L^{-1}	14.5 mg L^{-1}	> 100 mg L^{-1}
Propranolol	288.2 µg L^{-1}	90.4 µg L^{-1}	0.1 – 1 mg L^{-1}
Bezafibrate	~ 150 mg L^{-1}	-	10 – 100 mg L^{-1}
Trimethoprim	5.1 mg L^{-1}	2.4 mg L^{-1}	10 – 100 mg L^{-1}
Paracetamol	265.8 mg L^{-1}	93.4 mg L^{-1}	10 – 100 mg L^{-1}
Fluorene	3.99 mg L^{-1}	-	-
Anthracene	1.75 mg L^{-1}	-	-
Phenanthrene	1.18 mg L^{-1}	-	-
Pyrene	0.36 mg L^{-1}	-	-
Phenol	9.59 mg L^{-1}	-	-
4-chloro-3-methylphenol	7.91 mg L^{-1}	-	-

* Results of range finding tests

3.1.4.2. Exposure to environmental mixtures – Passive dosing

3.1.4.2.1. Development

The spiking experiment (see § 0) showed good results (Figure 15). Equilibrium was achieved after 1 hour and the expected concentration in the test medium was reached.

Results of the modeling experiments 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 16a). 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. The amount of sampler chosen in this study, was 3 g.
Figure 16b 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, t95%, varied from 3.3h for a log(Kd) of 3.8, to 4.5h for a log(Kd) of 5.6.

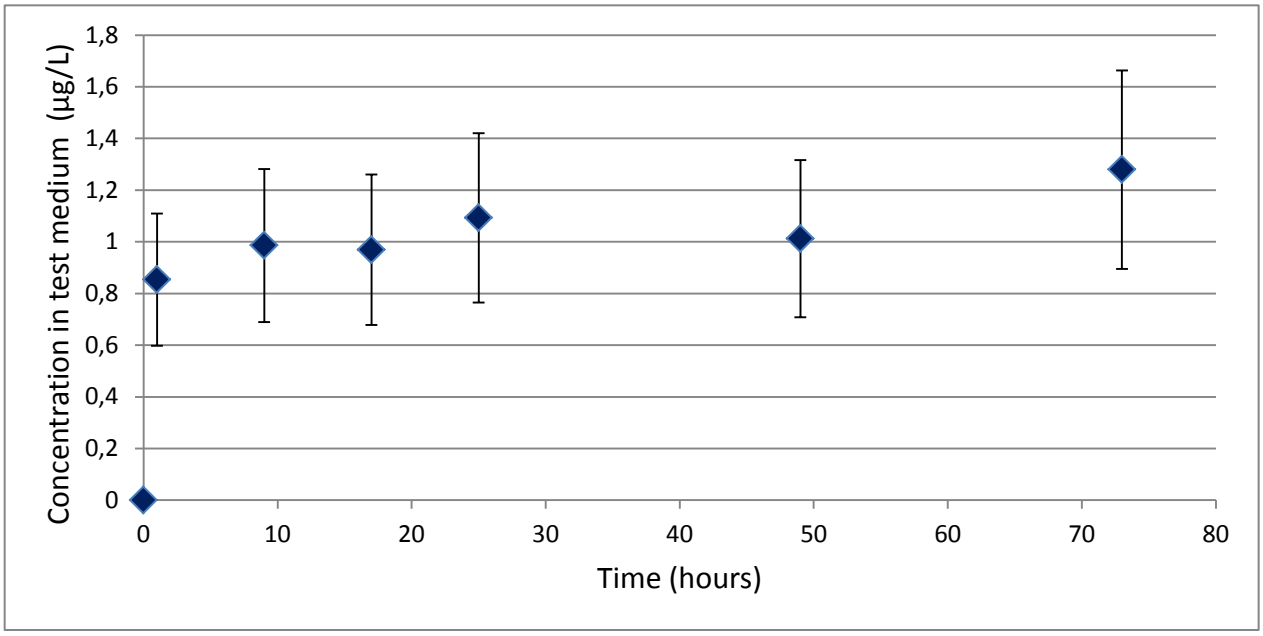

Figure 15. Results for fluoranthene of the equilibrium experiments with spiked passive samplers.

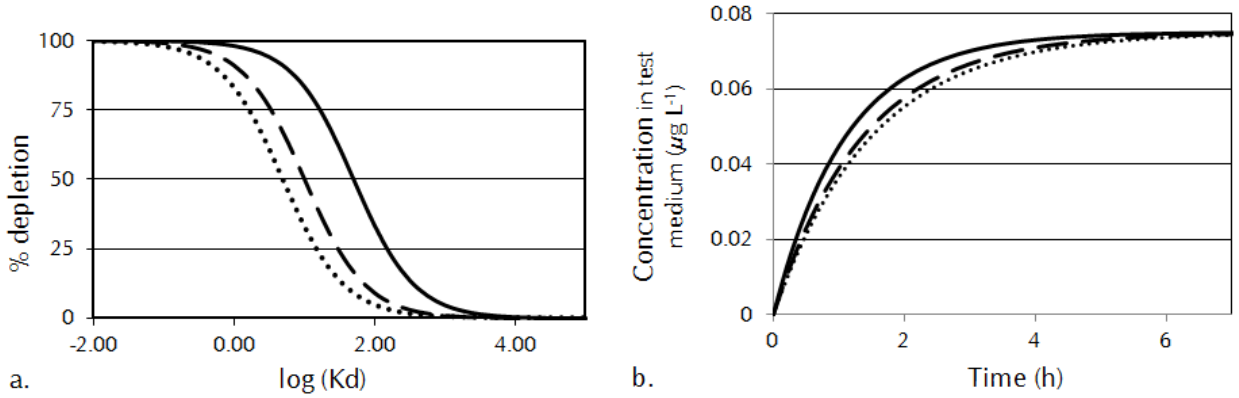

Figure 16. (a) Relation between % of depletion of the passive sampler and Log(Kd) of the absorbed compound, volume of the test medium was assumed to be 0.05L, the amount of sampler added was 1g (solid line), 5g (dashed line) or 10g (dotted line); (b) release kinetics of compounds with varying Log(Kd): fluorene (solid line; log(Kd) = 3.82), fluoranthene (dashed line; log(Kd) = 4.69), benzo(a)pyrene (dotted line; log(Kd) = 5.56).

3.1.4.2.2. Ecotoxicity testing

The results of the toxicity tests using contaminated passive samplers as a source of environmental pollutant mixtures showed different results between the samplers deployed in 2008 and 2009. Table XIX shows a summary of results for the *P. tricornutum* 72h growth inhibition test and the *C. gigas* 24h larval development test using the passive samplers deployed during the biomarker experiments in the autumn of 2008 and the spring of 2009, and during the sampling campaign of 2008.

Differences in toxicity can be noticed between the sampling locations and the sampling periods. For example, the mixtures desorbed from the samplers deployed in the autumn of 2008 (Biomarker 2008), clearly show the least toxic effects as the marine diatom exhibits growth stimulation for all sampling stations and some of the stations do not cause 100% abnormality in oyster larvae. The latter cannot be said for the other sampling periods for which 100% abnormality was observed for nearly all stations (only one exception).

Table XIX. Results of the *P. tricornutum* 72h growth inhibition test and the *C. gigas* 24h larval development test involving exposure to environmental micropollutant mixtures desorbed from field-contaminated passive samplers. PT: *P. tricornutum*; % GI: percentage growth inhibition after 72h of exposure; CG: *C. gigas*; % Abn.: percentage of abnormally developed (including dead) larvae after 24h of exposure.

Campagne 2008	ZB3	ZB2	OO1	OO2	OO3	NP1	NP2	R&V
PT - % GI	-27	100	100	68	-6	-8	-25	22
CG - % Abn.	100	100	100	100	100	100	100	100
Biomarker 2008	ZB3	ZB2	OO1	OO2	OO3	NP1	NP2	R&V
PT - % GI	-123	-124	X	-117	X	X	-119	X
CG - % Abn	61	80	X	100	X	X	98	X
Biomarker 2009	ZB3	ZB2	OO1	OO2	OO3	NP1	NP2	R&V
PT - % GI	100	-53	X	-53	X	X	-40	-63
CG - % Abn.	100	100	X	100	X	X	100	96

For *P. tricornutum*, an attempt was made to explain the observed toxicity of the mixtures by using a dose-addition approach (see §0). As the concentration data of the passive samplers themselves were limited to PCBs and PAHs, the concentrations measured via conventional sampling were used. To this end, the ranges of these data (obtained over the different sampling years) were used in a Monte-Carlo analysis to generate 100,000 sets of fictional concentrations per station for pharmaceuticals, organotins, pesticides, PAHs, PCBs, PBDEs, phenols, phthalates and PFCs (see §0). For each of the 100,000 sets the hazard index (HI) was calculated. The ranges of HI obtained in this fashion are presented in Figure 17. The highest HI was observed at station OO2, exhibiting a median value 0.12 of and a maximum value of 0.21. As such, this result cannot explain

the growth inhibition of 68% that was observed for the samplers from the sampling campaign in 2008 (Table XIX), as to obtain this inhibition rate HI should be higher than 1. The most likely explanation is that during the 2008 campaign other chemicals (highly) toxic to the test organism were present that were not measured in this project. It is unlikely that the concentrations of some of the chemicals included in the assessment was much higher in the test medium than what would be reflected by the measured environmental concentrations used for the HI calculation. Indeed, as passive samplers reflect time-weighted average concentrations, any peaks in environmental concentrations are flattened. Moreover, the passive sampling data reflect freely dissolved concentrations while the data obtained through conventional analysis of water samples are comprised of the freely dissolved fraction and the fraction bound to dissolved organic matter. Conventional analysis thus overestimates the freely dissolved fraction as measured by passive sampling (although in §3.1.2 it was illustrated that the discrepancy between the two methods is small). Thus, for future research, it will be important to increase the number of compounds that can be measured on this type of passive samplers.

In general, pesticides were the greatest contributor to the HI of the mixtures (as illustration, Figure 18 shows the contributions of the different chemical groups to the HI of the mixtures at station OO2). Only at station ZB2, PAHs were the greatest contributor.

For the test organism *C. gigas*, the results were generally more severe. However, due to a lack of ecotoxicity data for this organism, a mixture toxicity assessment could not be performed at this point.

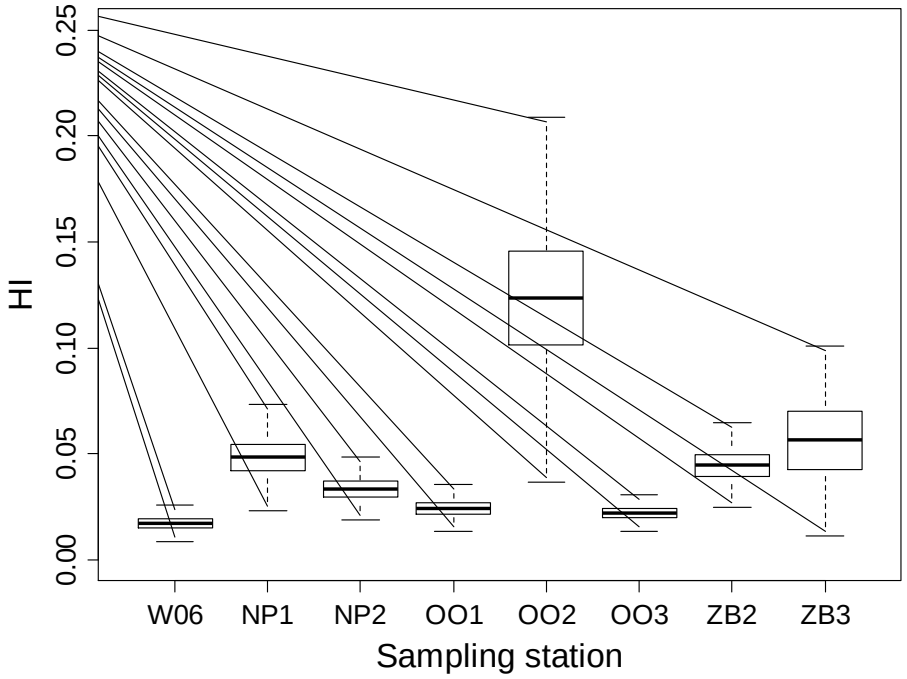

Figure 17. Boxplots of sum of toxic units (TU) obtained via Monte-Carlo analysis to generate 100,000 sets of fictional concentrations per station for pharmaceuticals, organotins, pesticides, PAHs, PCBs, PBDEs, phenols, phthalates and PFCs (see text).

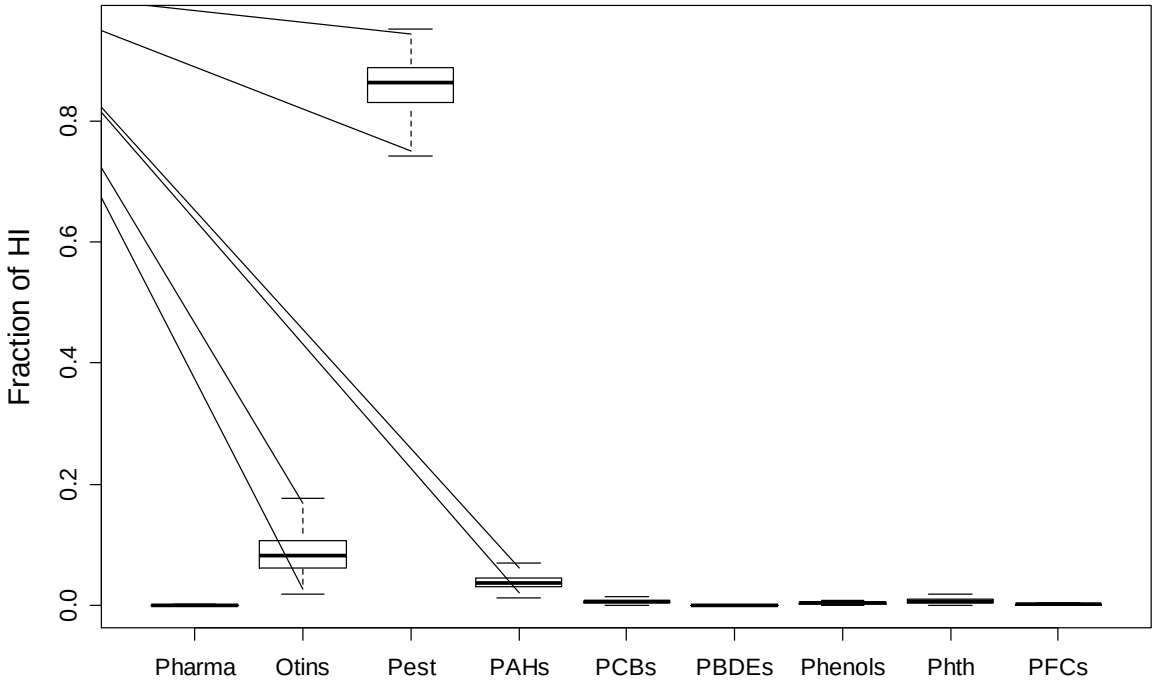

Figure 18. Contribution of the different chemical groups to the HI of the 100,000 mixtures as generated by Monte-Carlo analysis for sampling station OO2. Pharma: pharmaceuticals, Otins: organotins, Pest: pesticides, Phth: phthalates, HI: hazard index.

3.1.4.3. Biomarker experiments

For the biomarker experiments, caged mussels (*M. edulis*) were exposed to harbour waters and a control station at sea during six weeks (with simultaneous deployment of passive samplers for chemical analysis and ecotoxicological testing).

3.1.4.3.1. Cellular energy allocation and EROD

Results of the CEA biomarker show increased CEA values of *M. edulis* in the harbour of Nieuwpoort both in 2008 and 2009 compared to the control organisms (see Figure 19). In 2008, a slight decrease was noticed for the organisms deployed at the marinas of Oostende and Zeebrugge. The analysis of the EROD biomarker shows a significant increase of EROD activity (compared to the control organisms) in all harbours in 2008, with the exception of station ZB3 (see Figure 20). In 2009, both stations at Zeebrugge have significantly lower EROD values.

Table XX shows the correlation of the biomarker responses with the concentrations of PAHs and PCBs as derived from passive sampling. The correlation coefficients show that the EROD activity is positively correlated with the concentration of PCBs and more precisely the planar PCBs. The latter are the group of PCBs with the highest potential to induce EROD activity. That PAH concentrations do not show a significant correlation with EROD activity is rather surprising, as they are also known as EROD inducers.

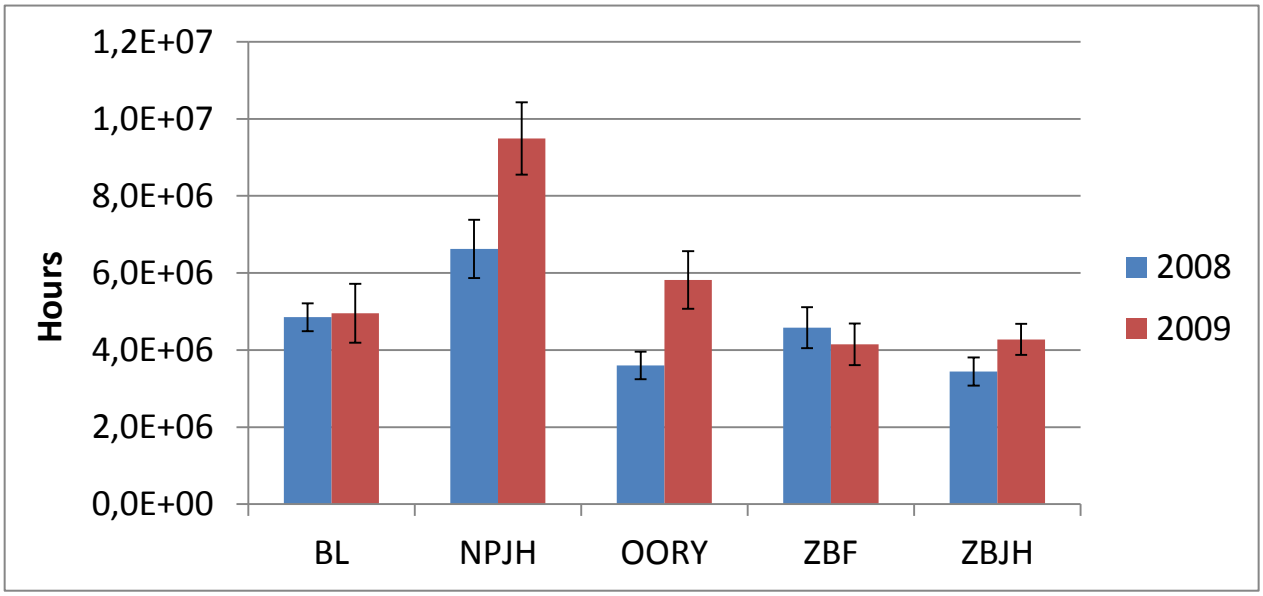

Figure 19. Results (in hours) of the cellular energy allocation analysis of mussel digestive glands after in situ exposure to harbour waters (+/- SD).

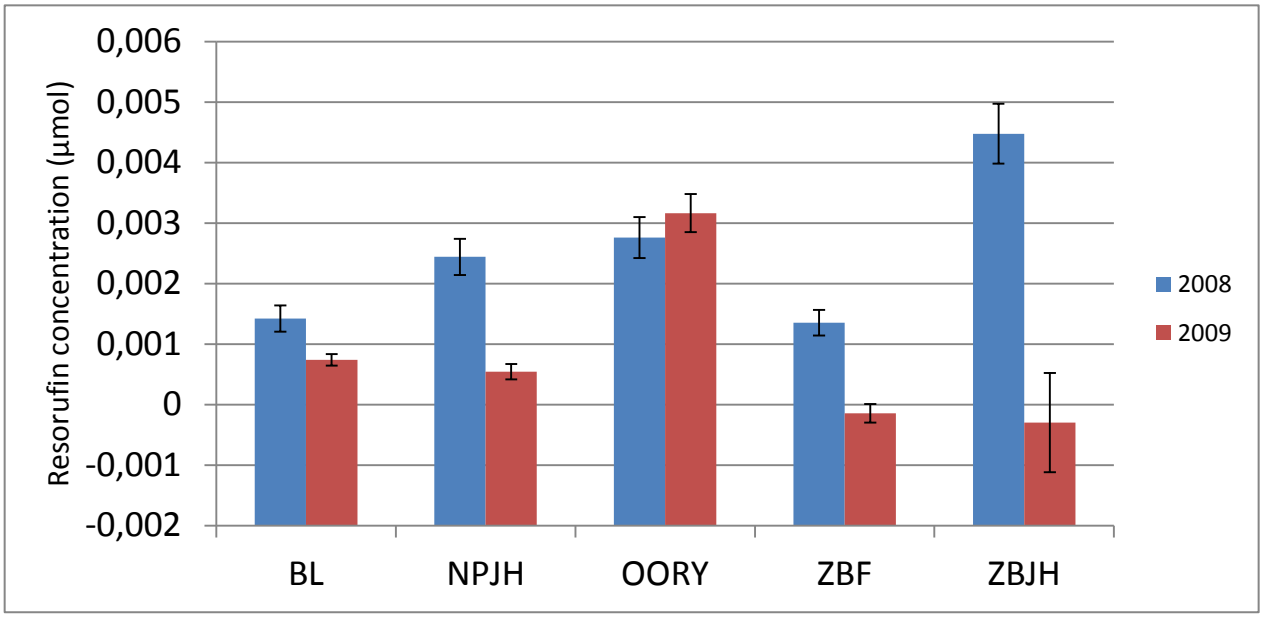

Figure 20. Results of the EROD analysis of mussel digestive glands after in situ exposure to harbour waters (+/- SD).

Both PAHs as PCBs showed a positive correlation with carbohydrate and protein levels and a negative correlation with lipid levels and rate of metabolims (ETS). This suggests that the stress due to pollution (not necessarily limited to PCBs and PAHs) causes on the one hand a reduction in metabolism, and on the other hand an increased energy use as suggested by the drop in long-term energy reserves (i.e. lipids) and an increase in short-term energy reserves (i.e. carbohydrates).

Table XX. Pearson correlation coefficients for the biomarkers EROD and CEA. PPCB: planar PCBs.

	EROD	Proteins	Carbohydrates	Lipids	ETS
SumPAH	0.114	0	0,346**	-0,292**	-0,267**
SumPPCB	0,627**	0,442**	0,360**	-0,223**	-0,198*
SumPCB	0,632**	0,302**	0,377**	-0,350**	-0,211*

3.1.4.3.2. Lysosomal membrane stability and ALP

The two short term transplantation experiments, one in the autumn of 2008 and one in the spring of 2009, revealed no clear differences between the stations on the level of biometric characteristics such as growth, condition indices and gonadal development. The Lysosomal Membrane Stability (LMS) showed lower mean neutral red retention times (NRRTs) in the marinas of Oostende (OO2) and Nieuwpoort (NP2) compared to

the reference situation in the Eastern Scheldt or at sea (W03'). This suggests a potential impact caused by micro-pollutants in these two harbours. The Alkali Labile Phosphate (ALP) results were very variable and a fine-tuning of this biomarker is needed for the future. The harbour of Nieuwpoort (NP2) had significantly higher ALP levels compared to the outer harbour of Zeebrugge (ZB1) after the autumn 2008 experiment. The experiment in the spring of 2009 revealed that male mussels in the marinas of Nieuwpoort (NP2) and Zeebrugge (ZB2) showed also higher ALP levels. The ALP results might suggest a potential effect of endocrine disruptors in those stations, but no correlation could be made at this point. The short term exposure experiments revealed significant results from the cellular biomarkers comparable to the results of the long term mussel cage experiments of 2007 and 2008.
The LMS biomarker in the short-term cage experiments with mussels could be suggested as an effective tool for short-term multi-biomarker monitoring experiments of the micropollutants along the Belgian coastal zone. However, the ALP analyses method should be refined in order to obtain more stringent results.

3.1.5. Ecological assessment

It was decided not to analyse the samples of April 2008 as they can't be compared with the other sampling periods. 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 mysid shrimp densities, measured in the hyperbenthic samples, were too low (see example below) to be sampled adequately for chemical analyses and for population characteristics. Based upon this knowledge it was decided not to analyze any other hyperbenthic samples.

3.1.5.1 Macrobenthos biodiversity

In general, for all sampling stations (W01-W06, S01 & S22, ZB1-ZB4, OO1-OO4, NP1-NP3), 70 species were found in the Van Veen samples of the integrated sampling campaign of May-July 2007, 80 species were found in the Van Veen samples of the integrated sampling campaign of December 2007 and 100 species were found in the Van Veen samples of the integrated sampling campaign of July 2009. In general, the

diversity indices (Shannon-Wiener diversity index (H'), Margalef's species richness index (d), Pielou's evenness index (J') and species richness (S)) indicate an increasing gradient in diversity and density from the inner harbours towards the coastal zone (Figure 16). The non-parametric Mann-Withney U test to compare two groups of samples shows that the diversity indices H', d and S are significant different between harbour stations and coastal stations. No significant difference is found for J'.

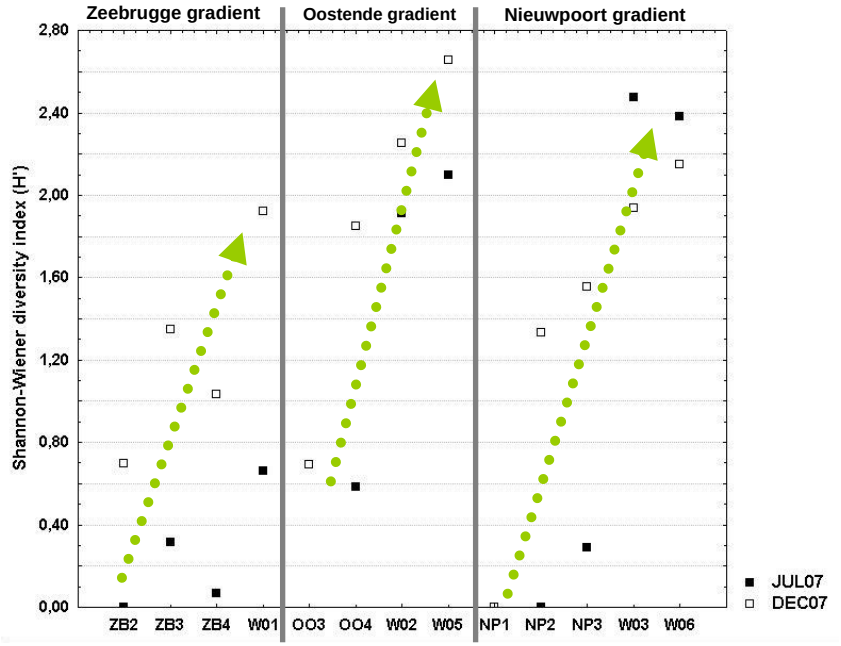

Figure 16. Macrobenthos Shannon Wiener diversity index (H') for all the sampled stations in July 2007 and December 2007

A MDS ordination on species abundances, of the macrobenthos samples from the July 2007 and December 2007 campaigns, with superimposed the abiotic variables shows that the harbour stations NP1 and NP2 are isolated from all the other stations (Figure 17). A sub MDS of the clustering stations reveals a clear spatial pattern in which the harbour stations are separated from the coastal zone stations. The superimposed abiotic variables vectors show an increasing gradient from the inner harbours towards the coastal zone for salinity and a decreasing trend in mud content, PAHs and O-tins and in December 2007 as well for PBDE's while PFOS shows a decreasing trend. Salinity (ρ = 0.51) explains best the macrobenthos abundances in JUL07 according to the BEST BIO-ENV analysis results, followed by salinity and PAHs (ρ = 0.43); salinity and PBDEs (ρ = 0.42), etc. Mud content, PAHs and PON (ρ = 0.44) explain best the macrobenthos abundances in DEC07 according to the BEST BIO-ENV analysis results, followed by salinity, mud content, PAHs and PON (ρ = 0.42); salinity, mud content, PAHs and POC (ρ = 0.41); etc. Significant ($p < 0.05$) non-parametric Spearman rank correlations were

found between S, d, H' and salinity, mud content, O-tins and PAHs in JUL07 and in DEC07. Significant correlations were as well found in DEC07 for S, PBDEs and PFOS; H' and PBDEs and POC.

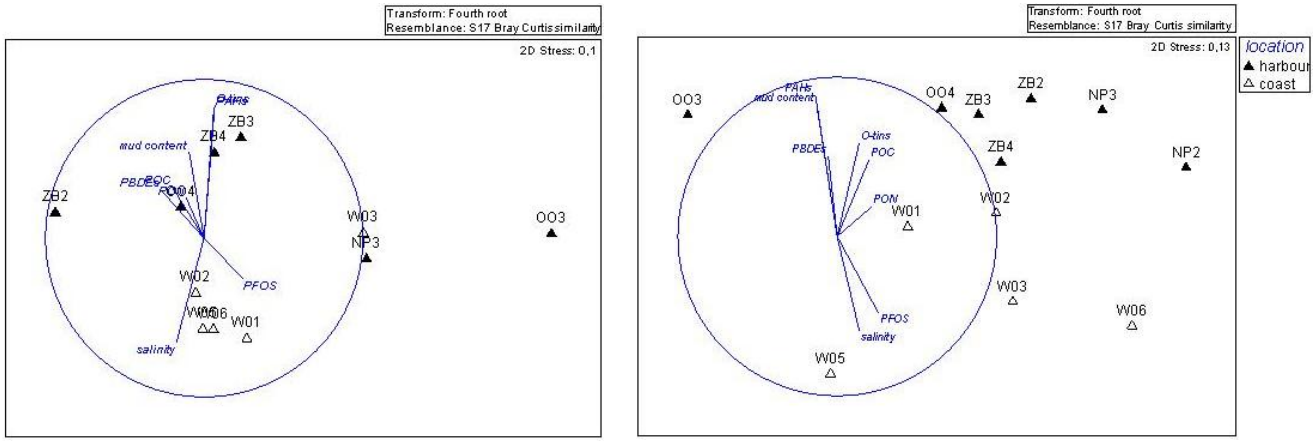

Figure 17. MDS ordination of macrobenthos species abundances of the integrated sampling campaigns of May-July 2007 and December 2007 with superimposed the vectors of the abiotic variables (salinity, mud content, PAHs, O-tins, PON, POC, PBDEs, PFOS).

Biodiversity of macrofauna increases significantly along the gradient from the inner harbours towards the coastal zone. A decreasing gradient of sediment contamination exists for O-tins, PAHs in DEC07 and JUL07; and for PBDEs in DEC07; an increasing gradient exists for PFOS. The biodiversity of the macrobenthos is influenced by plural abiotic factors such as mud content, salinity and pollutants.

3.1.5.2 Long term cage experiments

3.1.5.2.1. 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.

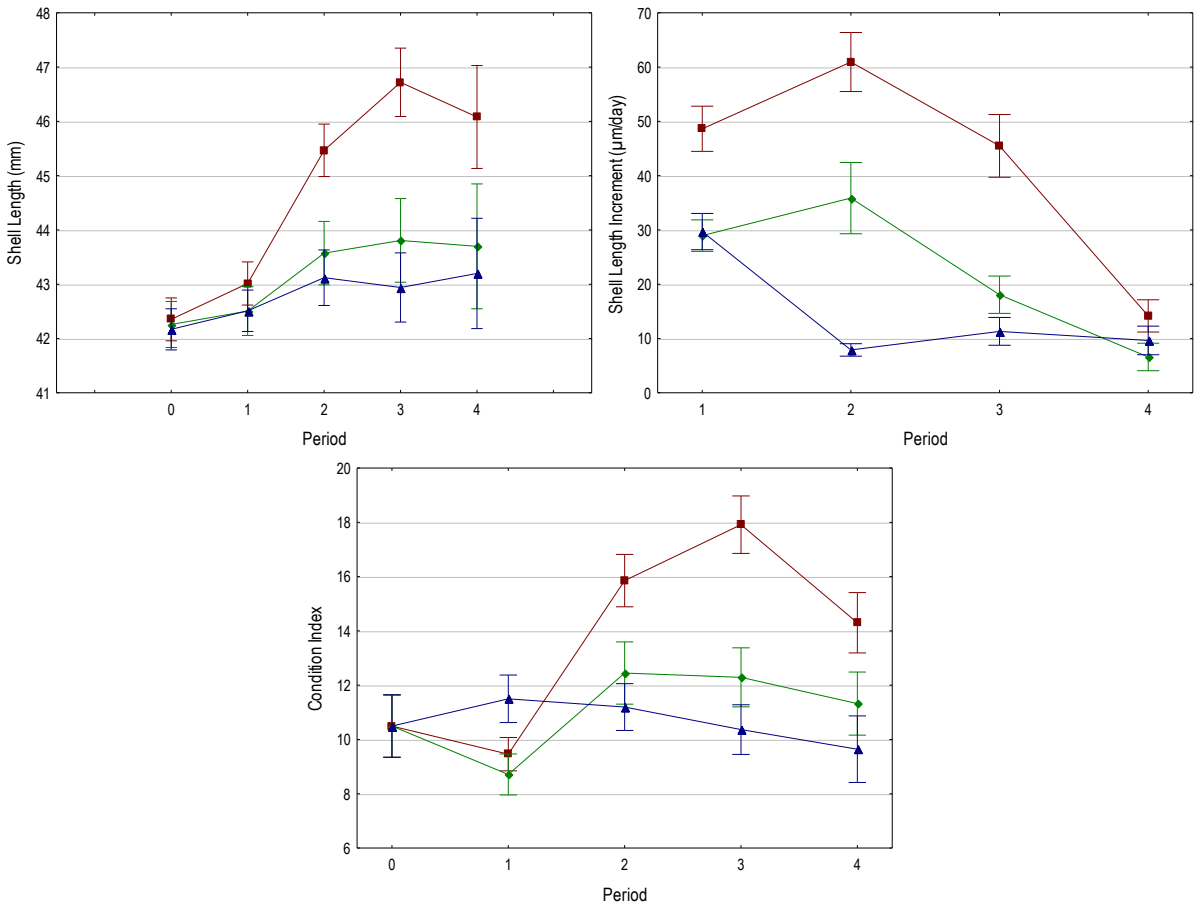

Figure 21. Shell Length, Shell Length Increment and Condition Index of Mytilus edulis Jun -Oct '07. Station ■ = ZB3, ▲ = ZB2, ◆ =OO1

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 gametogenic development of the mussels was in 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.

3.1.5.2.2. 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) (Figure 22). 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.

3.1.5.3. Population characteristics of Sterna hirundo

Common tern eggs couldn't be sampled during spring 2009 due to a very bad breeding success in the colony. Therefore, eggs were sampled in 2010. Overall, 32 common tern eggs were sampled for chemical analyses. Population data of the reproductive success and the development of the chicks originating from that common tern colony are currently being analysed.

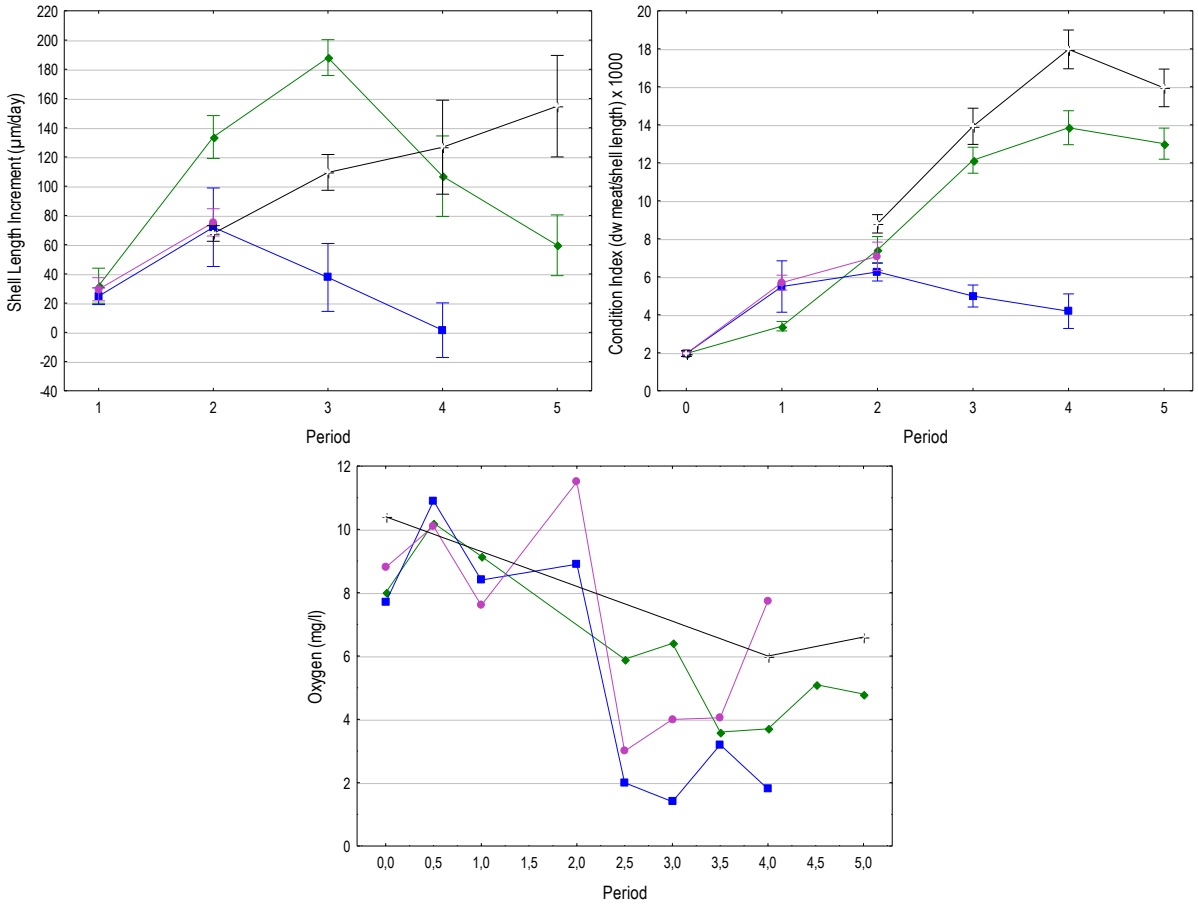

Figure 22. Shell Length Increment, Condition Index of Mytilus edulis and H2O Oxygen concentration Febr-Jul '08 Station + = W03', ◆ = ZB2, ■ = OO2, ● = NP2'

3.1.5.4. Population characteristics of mysid shrimps

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.

3.1.6. Integrated monitoring

Figure 3 in §2.1.7.1., shows a presentation of the integrated monitoring scheme explored in this project. In this scheme, passive samplers are used to estimate concentrations of micropollutants in all relevant marine compartments (i.e. water,

sediment, SPM and biota) and they function as a source of environmentally relevant pollutant mixtures in laboratory ecotoxicity tests, allowing to study the ecotoxicological characteristics of the mixtures. The data gained from these two steps, will be combined with information on the ecological health status of the north sea by performing ecological monitoring. To validate this integrated monitoring scheme, sampling campaigns were organized in which an optimal integration of all separate steps was envisaged. During these campaigns, samples of the marine compartments mentioned above, were collected for conventional chemical analysis. The sampled biota were studied both ecologically as chemically, yielding both body burden data and data on biodiversity. For example: 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 (Table XXI). 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.

Table XXI. 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

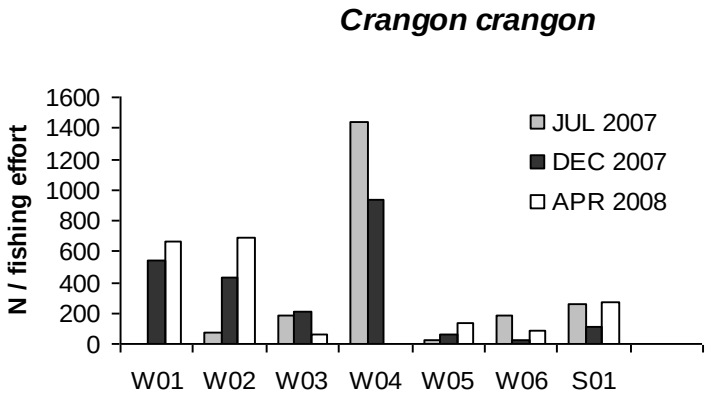

Figure 19. Number of *Crangon crangon* caught per fishing effort of sampling campaigns July '07, December '07 and April '07

C. crangon, was also sampled for chemical analyses (Figure 19). 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 relatively 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).

3.1.7. Integrated risk assessment

The risks of micropollutants to both the environment as to human health was assessed. For PAHs and PCBs this was done based on both conventionally measured data as for data obtained through the equilibrium modeling described in §0. For the tern population, no sound risk assessment could yet be performed.

3.1.7.1. Assessing effects on ecosystem health – Water and sediment compartment

The results of the risk assessment are summarised in Table XXII and Table . Data for a specific compound is only presented if the maximum calculated RCR was higher than 0.5 (water) or 1 (sediment) in order to show the most important results. For sediment, RCR values calculated using the modeled data from §0 are presented as well (only for PCBs and PAHs). Figure 23 shows a visual representation of the RCR values obtained using the measured and modeled data for the sediment compartment.

Table XXII. Ranges of RCR values of micropollutants in the water column as observed at offshore sea stations and in the coastal harbours. %RCR > 1 represents the percentage of cases in which the observed RCR exceeded 1, indicating a risk.

	RCR -- Sea				RCR -- Harbours			
Compound	min	median	max	%RCR>1	min	median	max	%RCR>1
Acenaphthene	0.001	0.008	0.127	0.0	0.001	0.032	2.598	2.4
Benzo(b)fluoranthene & Benzo(k)fluoranthene	0.023	0.160	1.530	15.4	0.003	0.515	3.067	33.3
Benzo(ghi)perylene & Indeno(1,2,3-cd)pyrene	0.007	0.073	0.627	0.0	0.030	0.203	1.533	2.4
Fluoranthene	0.005	0.017	0.058	0.0	0.010	0.045	0.507	0.0
Pyrene	0.010	0.031	0.108	0.0	0.015	0.091	0.763	0.0
Sum7PCBs	0.175	0.175	0.200	0.0	0.175	0.175	1.150	5.0
TBT	0.250	5.525	59.500	91.7	0.250	11.000	38.500	97.3
Propranolol	0.050	0.050	0.300	0.0	0.050	0.300	2.400	16.7
Bezafibrate	0.109	0.109	0.348	0.0	0.109	0.109	0.783	0.0
4-chloro-3-methylphenol	0.065	0.065	2.290	7.4	0.065	0.109	1.091	2.3
Bisphenol A	0.083	0.083	0.867	0.0	0.067	0.083	1.200	2.3
Dettol	0.139	0.139	0.139	0.0	0.139	0.139	1.389	7.0
Nonylphenol	1.515	1.515	1.818	100.0	1.515	1.515	33.636	100.0
Bis-(2-ethylhexyl)phthalate	0.008	0.081	1.419	14.3	0.008	0.008	0.766	0.0
Azoxystrobin	0.204	3.061	3.061	97.1	0.204	3.061	4.286	97.7
Carbendazim	0.310	0.310	0.310	0.0	0.116	0.310	1.240	2.3
chloridazon[1]	0.088	0.088	0.088	0.0	0.088	0.088	80.765	37.2
chloortoluron[1]	1.176	1.765	23.529	100.0	1.765	7.059	317.647	100.0
Diuron	0.015	0.015	0.840	0.0	0.010	0.040	2.270	4.7
Flufenacet	51.724	51.724	51.724	100.0	51.724	51.724	1500.000	100.0
Isoproturon	0.003	0.005	0.090	0.0	0.003	0.023	0.973	0.0
Linuron	1.250	1.250	1.250	100.0	1.250	1.250	318.750	100.0
metazachlor[1]	0.322	0.322	0.858	0.0	0.322	0.322	14.807	18.6
Metolachlor	0.150	0.150	0.200	0.0	0.100	0.150	74.000	20.9
Monolinuron	0.216	0.216	0.216	0.0	0.216	0.216	5.252	7.0
Pirimicarb	1.538	2.308	12.308	100.0	1.538	2.308	44.615	100.0
Propachlor	0.426	0.426	4.261	11.8	0.426	0.426	53.409	16.3
Terbutylazine	0.500	0.750	5.000	35.3	0.500	2.000	163.000	60.5
sum 7 PBDEs	0.150	1.238	101.500	57.7	0.150	3.175	73.300	78.8

[1]Quality criterion based on an incomplete dataset: results should be interpreted with caution

The RCR values for the water compartment presented in Table XXII show that for a number of compounds the quality criteria are exceeded, which happens most often in the coastal harbours. A number of PAHs exceeded 1 by a factor up to 3 and the highest frequency of exceedance occurred in the harbour of Zeebrugge. TBT exceeded its quality criterion for the water column up to a factor of 38.5 in the harbours and up to a factor of almost 60 at sea at a rate of nearly 100% in both cases. Of the pharmaceuticals, only propranolol posed a concern in the harbours of Oostende and Nieuwpoort. Of the phenols, nonylphenol posed the greatest concern, exhibiting a risk both at sea as in the

harbours with RCR values up to 34 for the latter. However, the fact that the RCR for nonylphenol exceeded 1 in 100% of the cases, was caused by the fact that the PNEC value of 0.033 μg L^{-1} was lower than the detection limit (0.1 μg L^{-1}). The same was true for the pesticides flufenacet, chlortoluron, linuron and pirimicarb. Among the pesticides, high concerns were observed for flufenacet, linuron, metolachlor, propachlor and terbutylazine, all having RCR values above 50. The same was true for chloridazon and chlortoluron, but this could be due to their highly conservative PNEC values which were based on incomplete datasets. However, the observed concerns warrant further investigation for these pesticides as well.

Table XXIII lists the RCR values for sediment. For this compartment, 13 PAHs posed a concern. The highest risk was observed for acenaphthene, which exhibited RCR values of up to 836 (at station ZB2 in 2008). This PAH also posed a risk at the sea stations with RCR values up to 56 at station S01. TBT posed the greatest concern for sediment with RCR values up to 14800 (OO1) and 3800 (S01) for harbour and sea stations, respectively. PCBs also posed a concern at both sea and harbour stations. PBDEs on the other hand, only exhibited a risk at sea at all nearshore stations (W01-W03).

Table XXIII. Ranges of RCR values of micropollutants in sediment as observed at offshore sea stations and in the coastal harbours. %RCR > 1 represents the percentage of cases in which the observed RCR exceeded 1, indicating a risk. M: based on measured data; P: based on predicted sediment concentrations using the equilibrium modeling results described in 2.2.1.3.

		RCR -- Sea				RCR -- Harbours			
Compound	M/P	min	median	max	%RCR>1	min	median	max	%RCR>1
Acenaphthene	M	7.81	15.63	56.25	100.0	18.75	67.81	836.25	100.0
	P	10.69	11.96	33.57	100.0	6.02	176.27	2573.70	100.0
Anthracene	M	0.03	0.22	0.58	0.0	0.23	0.76	8.48	34.1
	P	0.01	0.07	0.12	0.0	0.10	0.32	1.99	17.9
Benzo(a)anthracene	M	0.01	0.18	0.45	0.0	0.29	0.56	22.93	25.0
	P	0.02	0.06	0.16	0.0	0.09	0.60	9.54	45.8
Benzo(a)pyrene	M	0.01	0.25	0.64	0.0	0.32	0.67	25.44	25.0
	P	0.00	0.14	0.53	0.0	0.00	0.66	9.54	33.3
Benzo(b)fluoranthene	M	0.02	0.29	0.77	0.0	0.38	0.96	24.56	43.2
	P	0.01	0.36	1.59	25.0	0.05	1.16	8.10	54.2
Benzo(k)fluoranthene	M	0.01	0.15	0.42	0.0	0.22	0.50	14.28	20.5
	P	0.01	0.18	0.78	0.0	0.05	0.56	3.96	16.7
Benzo(ghi)perylene	M	0.02	0.44	0.79	0.0	0.26	0.80	12.51	31.8
	P	0.03	0.14	0.24	0.0	0.27	0.72	5.19	35.7
Indeno(1,2,3-cd)pyrene	M	0.02	0.64	1.70	29.6	0.39	1.34	22.17	84.1
	P	0.13	0.31	0.49	0.0	0.51	1.07	9.54	57.1
Chrysene	M	0.01	0.29	0.61	0.0	0.33	0.71	22.30	25.0
	P	0.06	0.18	0.24	0.0	0.08	0.75	8.64	37.5
Fluoranthene	M	0.01	0.08	0.32	0.0	0.10	0.41	12.84	20.5
	P	0.11	0.17	0.34	0.0	0.07	0.65	12.05	39.3
Naphthalene	M	0.08	0.62	1.96	25.9	0.56	1.75	18.02	68.2

	P	--	--	--	--	--	--	--	--
Phenanthrene	M	0.06	0.30	0.74	0.0	0.34	0.88	10.35	38.6
	P	0.54	0.65	1.42	25.0	0.19	0.94	9.72	50.0
Pyrene	M	0.03	0.51	1.38	14.8	0.62	1.62	21.98	90.9
	P	0.26	0.98	2.97	50.0	1.24	5.57	62.38	100.0
Sum 7 PCBs	M	0.35	14.08	20.00	44.4	17.50	17.50	77.00	100.0
	P	8.87	44.85	149.66	100.0	18.07	113.40	544.89	100.0
TBT	NA	--	1900.00	3800.00	--	1920.00	3200.00	14800.00	100.0
Sum 6 PBDEs	NA	0.00	0.01	2.65	15.0	0.00	0.01	0.02	0.0

The risk assessment of PAHs and PCBs in sediment was also performed using predicted sediment concentrations based on passive sampling data (see 0). The data hereof are included in Table XXIII and are graphically presented in Figure 23. As can be seen in Figure 23, the median RCR values obtained via both methods correspond very well. The maximum values are generally within a factor 3. This is very good result, which makes the potential application of passive samplers in this way an attractive option. Indeed, as the analysis of passive samplers is much less complex and much cheaper than the conventional analysis of sediment samples, it could provide an alternative to perform routine monitoring of sediment quality.

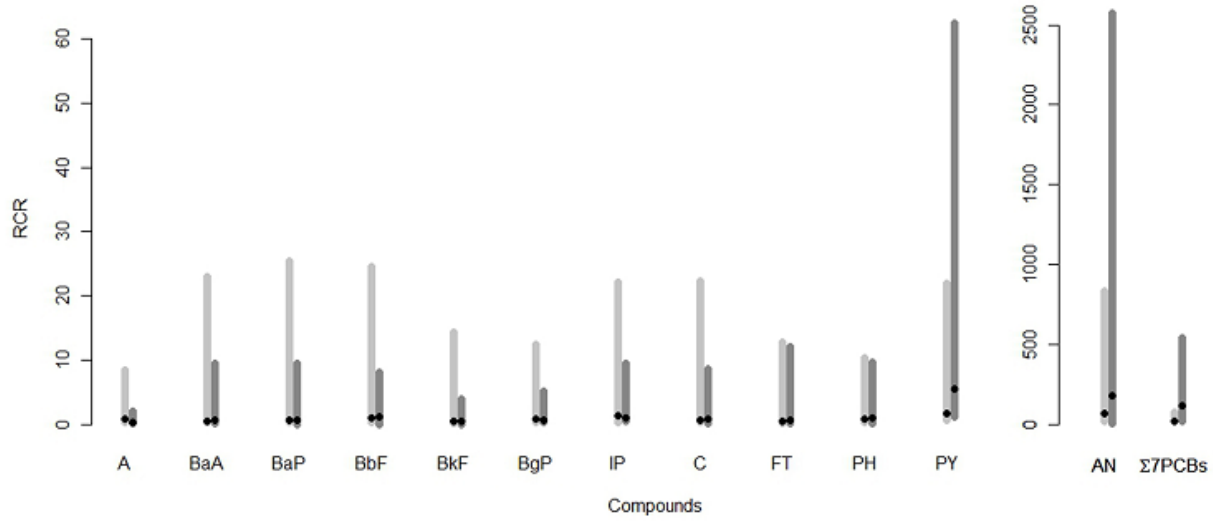

Figure 23. Comparison of measured and predicted RCR values of PAHs and PCBs in sedi-ment of the harbours. The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respec-tively. Black dots represent the median values. AY: acenaphthylene, A: anthracene, BaA: benzo(a)anthracene, BaP: benzo(a)pyrene, C: chrysene, DA: dibenz(a,h)anthracene, FT: fluoranthene, F: fluorene, PH: phenanthrene, PY: pyrene, AN: acenaphthene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoran-thene, BgP: benzho(g,h,i)perylene, IP: indeno(1,2,3-cd)pyrene, sum7PCBs: sum of PCB congeners 28, 52, 101, 118, 138, 153 and 180.

3.1.7.2. Assessment of secondary exposure of predators and humans indirect via the environment

The RCR values for secondary poisoning to predators of micropollutants in bivalves are presented in Table XXIV (only the chemicals posing a concern are shown). Of the PAHs, fluoranthene and pyrene posed a concern, but only in the harbours. The same was true for PFOS, which only posed a risk once at station ZB3. PCBs on the other hand, exhibited risk both in the harbours and at sea, achieving an RCR of nearly 80 at station OO1.

Table XXIV. Ranges of RCR values for secondary poisoning to predators of micropollutants in bivalves as observed at offshore sea stations and in the coastal harbours. %RCR > 1 represents the percentage of cases in which the observed RCR exceeded 1, indicating a risk. M: based on measured data; P: based on predicted sediment concentrations using the equilibrium modeling results described in §**3.1.3.1 Sediment and SPM**.

		RCR -- Sea				RCR -- Harbours			
	M/P	min	median	max	%RCR>1	min	median	max	%RCR>1
Fluoranthene	M	--	--	--	--	--	--	--	--
	P	0.01	0.02	0.06	0.0	0.02	0.11	1.56	3.8
Pyrene	M	0.03	0.07	0.12	0.0	0.08	0.38	5.82	30.6
	P	0.00	0.02	0.06	0.0	0.01	0.08	0.74	0.0
sum7PCBs	M	3.88	5.96	6.67	100.0	7.61	24.08	79.10	100.0
	P	0.35	1.81	6.15	83.3	0.78	5.74	21.73	96.2
PFOS	NA	0.00	0.01	0.08	0.0	0.00	0.15	1.15	2.6

Table XXV shows the RCR values obtained for human health through the consumption of different marine food sources. Only the chemicals posing a concern are shown, which were PCBs, benzo(a)pyrene (the representative compound for the other PAHs), dichlorvos and organotins. The greatest risk was posed by PCBs, which exhibited RCR values up to 12.5 and 3.5 at harbour and sea stations, respectively.

Table XXV. Ranges of RCR values for secondary poisoning to humans of micropollutants in different marine food sources as observed at offshore sea stations and in the coastal harbours. %RCR > 1 represents the percentage of cases in which the observed RCR exceeded 1, indicating a risk. M: based on measured data; P: based on predicted sediment concentrations using the equilibrium modeling results described in §**3.1.3.1 Sediment and SPM**.

			Sea				Harbours			
Compound	Food source	M/P	Min	Median	Max	%RCR>1	Min	Median	Max	%RCR>1
SumPCBs	S	M	0.336	0.434	0.930	0.0	--	--	--	--
		P	0.109	0.593	1.733	25.0	0.226	1.358	6.232	57.1
	B	M	0.575	0.996	1.140	55.6	0.733	3.864	12.475	93.2
		P	0.274	1.487	4.350	75.0	0.568	3.409	15.640	92.9
	F	M	0.781	1.312	3.480	80.0	--	--	--	--

		P	0.335	1.820	5.324	75.0	0.695	4.172	19.142	92.9
Benzo(a)pyrene	S	P	0.000	0.013	0.049	0.0	0.000	0.078	1.131	4.2
	B	P	0.001	0.017	0.063	0.0	0.000	0.066	0.875	0.0
	F	P	0.002	0.074	0.274	0.0	0.002	0.339	4.920	25.0
Dichloorvos	B	NA	0.000	0.135	0.222	0.0	0.003	0.144	0.690	0.0
	S	NA	0.030	0.151	0.217	0.0	--	--	--	--
	F	NA	0.002	0.133	0.211	0.0	--	--	--	--
SumOrganotins	B	NA	0.050	0.060	0.100	0.0	0.088	0.319	1.529	12.9
	S	NA	0.130	0.245	0.325	0.0	--	--	--	--

Table XXIV and Table XXV also show the RCR values obtained through the use of modeled data (see 2.2.1.3). As there are not as many data points to compare as for sediment, a profound comparison is not possible. However, the RCRs based on measured and predicted data were generally very close. Moreover, for fish and shrimp, which could not be caught in the harbours as elaborate fishing was not possible there, passive sampling allowed for a risk assessment of these organisms in the harbours as well. While the need for such a risk assessment may not be that great within the harbours (generally, organisms there are not caught for human consumption), this does nicely illustrate the place passive sampling could have in future monitoring schemes. Indeed, passive samplers could be used as the standard monitoring approach, being easier and much cheaper than conventional methods, and could be used to trigger conventional monitoring only when it indicates a risk. If this technique develops further, even the conventional monitoring may eventually be no longer necessary. However, more research into the reliability of the use of passive samplers in this fashion is certainly necessary.

3.1.8. Concluding remarks

From this final report it is clear that all main goals of the project were achieved and the project can thus be considered a major contribution in the assessment of the environmental quality of Belgian coastal waters and in the development of new, innovative techniques and approaches to assess, monitor and manage marine ecosystems. The main achievements can be summarized as follows:

- Novel analytical methods were developed and existing methods were improved during the course of this study.
- They have allowed obtaining an idea about the presence and distribution of a suite of hazardous substances, some previously undetected, in the major ports and along the Belgian coast.
- Passive sampling has proven to be a valid alternative to classical monitoring and a promising dosing tool for effects monitoring.

- Effects monitoring (in situ and in vivo) shows distinct differences between the harbours, validating it's use in an integrated monitoring scheme.
- There is a clear difference in the abundance and distribution of species between the investigated area's. This could partly be related to pollution illustrating the need to include ecological monitoring in an integrated scheme.
- Clear progress was made in the risk assessment of the substances under investigation.
- Including consumer species and top predators in the sampling, allowed assessing the risks for the highest trophic levels, including man.
- Distribution of contaminant between the compartments through the dissolved phase (simulated by passive samplers) has proven to be a valid working hypothesis, predicting the presence and distribution of hazardous substances in the marine environment.
- The project has laid the foundations for integrated monitoring and risk assessment in the Belgian coastal area.

4. POLICY SUPPORT

Belgium has the obligation to assess the status of its coastal area in compliance with the EU Water Framework Directive (WFD) and the International Convention on the Protection on the North East Atlantic or the OSPAR convention. Furthermore, the recent Marine Strategy Framework direction (MSFD) will require Belgium to assess weather it's coastal and marine waters have a good environmental status (GES). Both the WFD and MSFD further require members states make provisions to achieve a good environmental status by 2020 if this is not the case.

Central for these assessments, is the availability of quality data provided from monitoring programmes. Monitoring is one of the most important management tools used for assessing the quality status of an area and changes thereoff i.e. temporal trends. When monitoring for hazardous substances started, biota (fish and shellfish tissue) was the primary matrix of interest. This clearly stemmed from the focus on potential hazards to human health from the consumption of this seafood. Soon, other compartiments such as sediment and water were taken up in routine monitoring as well. Assessing the 'impact' of hazardous substances has been, and still is, based primarily on measurements of concentration. Obviously, because the questions being asked concerns concentrations of such substances in water, sediment and biota and, because such measurements are possible. However, over the past 40 years the objectives of monitoring have changed into an ecosystem approach where – in Europe under the WFD and MSFD – Good Environmental Status shall be reached. This Ecosystem Approach, adopted by the Joint HELCOM/OSPAR Ministerial Meeting in 2003, led to a different type of assessment. Objective now was to assess the pressures of human activities as well as the resulting impacts on, and state of, the marine environment. For hazardous substances, the goal is to obtain a comprehensive risk assessment for man and the environment.

In response to this more holistic demand and from a purely scientific perspective, there is general agreement that the best way to assess the quality status of the marine environment, with respect to hazardous substances, is by using a suite of chemical and biological measurements in an integrated fashion. In this respect, biological effects techniques have become increasingly important in recent years. Furhtermore, passive samplers have gained prominence among the novel techniques that attempt to assess the status of a given environment with regard to hazardous substances. Furthermore, not all adverse effects will be observed solely by looking at concentrations of contaminants or their known effects. One needs to establish a good knowledge about the presence and

distribution of species in any given enviroment hence the need for biological monitoring.

The WFD has embraced this ecosystem approach by introducing a lot of elements of it into the monitoring it foresees. Yet, biological effects monitoring is missing and the concentrations of most contaminants should only be determined in total water. OSPAR, on the other hand, has embraced the validity of biological effect monitoring and is currently developing guidelines for integrated monitoring. Both recognise the use of passive samplers as a valid approach although it has not yet been given a proper place in their schemes. These principles have also found their way into the MSFD but no clear guidelines are presently available. One can expect OSPAR to take the lead in this for the North Sea but this process is only starting. In any case, true integrated monitoring is not a fact yet.

This project aimed to take the next step in developing true integrated monitoring in Belgian coastal waters by looking at the different levels mentioned above and, by using both conventional and novel approaches. As such, it can support Belgian policy makers in their obligations of assessing the quality status of our waters as well as boosting research in this field. The outcome is also highly relevant for progressing intergrated monitoring at the international level i.e. OSPAR and MSFD level.

5. DISSEMINATION AND VALORISATION

The bulk of the results will be submitted for publication in peer reviewed journals. Published papers and papers in preparation are presented in § 6. The number of citations will be indicative for their impact. During the course of the project, poster and oral presentations were given at various conferences (e.g. SETAC conferences, VLIZ young scientist's day, etc.).

The results will further be presented at national and international fora (workshops, symposia, international working groups, etc.). Citations in reports, follow up in work programmes, requests for papers and further information, requests for short papers and/or text for proceedings, request for presentations on the subject, requests for collaboration, invitations to specialized working groups will indicate the impact of this study.

The data will be made available for national assessments of the quality status of Belgian waters and the North Sea as a whole. Use of and/or reference to the data will be indicative for its impact.

The results will be made available in an appropriate form to the general public (e.g. websites, popular magazines, presentations,..). Reactions from the general public (e.g. including questions from concerned citizens, non-profit organisations, the press) will be indicative for the impact.

6. PUBLICATIONS

Peer review

Claessens M., Monteyne E., Roose, P. and Janssen C.R. (in prep.) Development of a passive dosing technique to expose marine organisms to relevant micropollutant mixtures in laboratory ecotoxicity experiments.

Claessens M., Wille K., De Brabander H.F., Vanhaecke, L. and Janssen C.R. (in prep) Ecotoxicity of pharmaceuticals to the marine diatom Phaeodactylum tricornutum and the marine bivalve Mytilus edulis.

Claessens M., Rappé K., Monteyne E., Wille K, Roose P., Vincx M. and Janssen C.R. (in prep) Estimating the impact of environmental pollution with short term in situ experiments with Mytilus edulis.

Monteyne, E., Claessens M., Roose P., Janssen C.R. et al. (in prep.) Silicone Rubber Passive Samplers used in integrated monitoring of hydrophobic organic contaminants

Monteyne, E.; Wille K.; Roose P. et al. (in prep.) Distribution of organic contaminants the Belgian Coastal Zone: a 4-year survey.

K. Wille, H. Noppe, K. Verheyden, J. Vanden Bussche, E. De Wulf, P. Van Caeter, C.R. Janssen, H.F. De Brabander and L. Vanhaecke (2010) Validation and application of an LC-MS/MS method for the simultaneous quantification of 13 pharmaceuticals in seawater. Anal Bioanal Chem 397:1797–1808.

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Rappé K., Monteyne E., Wille K., Vincx M. et al. (in prep.) Blue mussels and pacific oysters in Belgian coastal harbours as indicators for environmental stress

Rappé K., Coates D., Claessens M., Monteyne E., Wille K., Vincx M. et al. (in prep.) In situ short term experiments with Mytilus edulis as indicators for environmental pollution

Others

Claessens, M., Monteyne, E., Roose, P., Janssen, C.R. (2008). The use of integrative passive samplers as a source of contaminant mixtures in ecotoxicological laboratory experiments, in: Mees, J. et al. (Ed.) (2008). VLIZ Young Scientists' Day, Brugge, Belgium, 29 February 2008: book of abstracts. VLIZ Special Publication, 40: pp. 36. Poster presentation.

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