The use of passive samplers as a central tool in integrated environmental risk assessments

Michiel Claessens

UNIVERSITEIT GENT

FACULTEIT BIO-INGENIEURSWETENSCHAPPEN

Voor Emmy en Wannes

Promotor:
Prof. Dr. Colin Janssen

Department of Applied Ecology and Environmental Biology
Laboratory of Environmental Toxicology and Aquatic Ecology

Dean:
Prof. Dr. Ir. Guido Van Huylenbroeck

Rector:
Prof. Dr. Paul Van Cauwenberge

Michiel Claessens

The use of passive samplers as a central tool in integrated environmental risk assessments

Thesis submitted in fulfillment of the requirements for the degree of
Doctor (PhD) in Applied Biological Sciences:
Environmental Technology

Dutch translation of the title:
Het gebruik van passieve monstername als centraal instrument in geïntegreerde milieurisico-evaluaties.

Reference:
Claessens, M., 2013. The use of passive samplers as a central tool in integrated environmental risk assessments. Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences, Environmental Technology, Ghent University, Ghent, Belgium.

ISBN-number:

RESEARCH GROUP
ENVIRONMENTAL TOXICOLOGY

This study was conducted as a part of the INRAM project (www.vliz.be/projects/inram), which was funded by the Belgian Federal Science Policy Office (BELSPO).

The experimental work was performed at the Laboratory of Environmental Toxicology and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University.

Dankwoord

Eind augustus 2006. Graag was ik – om de sfeer te scheppen – begonnen met een impressie van het weer, maar dat herinner ik me niet precies. Misschien was het zonnig en warm met kans op onweer naar de avond toe. Dat valt wel vaker voor in die tijd van het jaar. Wel herinner ik me nog levendig de vraag waar ik een antwoord op moest zoeken: begin ik aan een doctoraat... of niet? Mocht dit niet het dankwoord van mijn doctoraatsthesis zijn, dan had ik de spanning misschien nog even erin kunnen houden. Helaas!

Nu, juni 2013, ligt het eindresultaat er. Het was een lange en vaak zware weg, zeker het laatste jaar om met een nieuwe, voltijdse job en een kersverse baby die finale schrijfsels op papier te krijgen. Mocht ik er helemaal alleen voorgestaan hebben, dan was ik nooit tot hier geraakt. Ik ben ervan overtuigd dat dit laatste de realiteit is voor alle doctorandi. Dit deel is dan ook speciaal voor al diegenen die op één of andere manier mee hebben bijgedragen tot het tot stand komen van dit document. En jullie zijn met velen! Voor de gelegenheid heb ik dit dankwoord geïllustreerd met enkele cartoons van "*Piled higher and Deeper*"(PhD)[1]. Enerzijds omdat wetenschap niet altijd droog en humorloos hoeft te zijn, maar vooral ook omdat de realiteit – die cartoonisten veelal met angstwekkende precisie weten weer te geven – toch geregeld van de tekening afweek, wat ook vaak jullie verdienste was. Vandaar vind je hier vooral cartoons die niet van toepassing waren.

Professor, Colin, de persoonlijke transformatie die je tijdens een doctoraat ondergaat, is werkelijk uniek! En die was niet mogelijk geweest mocht ik niet de kans gekregen hebben bij u te doctoreren. Ook je veeleisendheid en vele verbeterwerk zijn hier tot het einde toe een hoofdfactor in geweest. Maar vooral je combinatie van voldoende vrijheid geven en toch op één of andere manier alles nagenoeg ongemerkt blijven volgen, was cruciaal. Deze situatie deed zich dan ook nooit voor:

[1] "Piled higher and deeper"by Jorge Cham, www.phdcomics.com

En dat is een luxe. Bedankt voor alles, inclusief de vlotte brainstorms!

Plateau-collega's! Het was een leuke tijd die ik me nog van het begin herinner. Karen, merci voor je fijne gezelschap in onze eerste doctoraatsmaanden in de kelder. Van opperste concentratie in absolute stilte tot gezellige babbel tijdens onze eigen koffiepauzes daar beneden. *And Giovanni (master of the oyster larvae), also a fellow basement-inhabitant for a while, thinking back how you were able to startle us when greeting your friend on Skype with a loud "Ola!" still makes me laugh! And I agree, spaghetti number 5 is the best!*
Michiel V. en Roel, mijn meest trouwe bureaumaten van het eerste verdiep! Op de duur wisten we in ons onderbewustzijn wiens beurt het was om de deur open te doen wanneer de bel ging of om de telefoon op te nemen.

Nog een niet te vergeten bureaumakker: Dieter! Je was direct mee met de toch wel bizarre humor die in onze ruimte heerste toen je aankwam. Frederik, R heeft nu

geen geheimen meer voor mij! Was jij trouwens niet degene die die bizarre humor in ons bureau heeft binnengebracht? Of was dat de droge humor? Meer niet te vergeten ecotox-mensen: Marlies, Brita, Lien (*I still feel the effect of that green tea*), Tu, Jana, en Karel. Jullie waren stuk voor stuk fijne collega's! Marianne, is er een administratief kluwen dat jij niet kan ontwarren? Beste Aeco- en CES&T-collega's! Pieter (ooit zal je het licht zien en een Nikon kopen), Koen (ik denk dat ons dak niet meer lekt!), Ine, Gert, Rob, Martine, Sigrid, Veerle, Sylvie en Peter: het eerste en tweede verdiep zouden zo gezellig niet geweest zijn zonder jullie!
Maar wat zouden die verdiepingen zijn zonder het gelijkvloers? Guido, eerste opperhoofd van het laboratorium, met glans opgevolgd door Nancy! Gisèle en Leen, ik kan me het labo niet voorstellen zonder jullie. Marc, kan iemand een proefopzet bedenken die jij niet kan bouwen?

Ook alle INRAM-collega's verdienen een bloemetje! Karen, ik ben er zeker van dat de campagnes op zee een stuk minder vlot waren verlopen als we het zonder jouw ENDIS-RISKS ervaring en efficiënte aanpak hadden moeten stellen. Klaas (mede-duizendpoot op het dek), Els, Annelies en An, bedankt voor de vlotte samenwerking. Mijn dank gaat ook uit naar de andere project-promotoren Patrick, Hubert, Lynn, Magda en Jan. En niet te vergeten, Gijs, Daniel en iedereen die op het VLIZ op de achtergrond hebben meegedraaid! Graag bedank ik ook alle medewerkers van de "plastiekprojecten": Eric, Ann, Elien, Hannelore en Valérie.

Ik wens ook alle studenten te bedanken die ik heb mogen begeleiden en die allemaal hun steentje hebben bijgedragen: Peter, Dries, Nele, Steven (microplastics pionier), Lieselot, Jasmien, Maarten, Saïda, Andy, Bart, Colette, Pleun, Sofie... en Lisbeth, ik wens je alle succes toe met je onderzoek naar microplastics! Ik ben er zeker van dat je dat tot een heel goed einde zal brengen.

My new DuPont colleagues! Lo, a very special thanks to you for your continued support, even when it became clear that finishing my PhD was going to take longer than anticipated. And I wish to extend that gratitude to Alexandra and Tineke. Laurent and Sébastien, the life on our island is great! Wendy, Françoise and Joeri, the Mechelen KG4-team would not be complete without you!

In je vrije tijd je batterijen weer opladen, doe je ook niet alleen... Spelletjesteam (Jill, Pascal, Dieter, Leen, Frédéric en Jan)! Die avonden en weekends gevuld met bikkelharde competitie en junk-food waren een ideale ontspanning! Laten we die vooral verderzetten!
Bart, er is geen plek waar je beter kan bezinnen dan Spitsbergen! Ik ben er zeker van dat ik daar nooit geraakt was zonder jouw zin voor avontuur. En laat me bij deze ook meteen de hele richting Biologie Antwerpen bedanken voor de fijne studententijd: Manu, Bram, Hans, Yves, Cleo, Sofie, Lise en vele anderen!
Ook een speciaal woordje van dank voor mijn pianoleraar Stefan! Een uurtje piano spelen kan werkelijk wonderen doen wanneer je even moet uitblazen van het vele werk. Bedankt om mijn pianospel op een behoorlijk niveau te krijgen!

En ten slotte over naar de familie. Mama, papa, jullie steun komt werkelijk vanuit alle mogelijke hoeken! Van de mogelijkheid om in alle vrijheid mijn studies te kiezen, over familie-feestjes die alle zorgen even doen vergeten tot morele, praktische en financiële hulp voor vanalles en nog wat, en dat tot in het verre Zwijnaarde. En papa, ik denk dat er van mijn doctoraat intussen meer backups zijn dan van alle andere doctoraten samen, en dat kan zelfs je leven redden:

Saar, Katleen en Elien, mijn drie geweldige zussen! Het was super om met jullie op te groeien. Ik hoop dat we ook nog veel lol kunnen trappen in de toekomst. Met die drie schoonbroers erbij kan dat geen probleem zijn. En Edward, ik zal onze dolle rit naar Brussel om mijn IWT voorstel op tijd in te dienen niet gauw vergeten! Emile (mijn petekind), Amélie en Marie, ik kijk er naar uit jullie verder te zien opgroeien.

Nonkel Hugo en Ann, mijn fascinatie voor de natuur komt voor een groot deel van bij jullie! Nonkel Yves en Tante Nadine, over een motorische vierwieler beschikken vanaf je 18de is een onvoorstelbare luxe. Jullie mobiliteitsgarantie is ongeëvenaard! Mami en Raymond, bedankt voor de interesse die jullie altijd getoond hebben in mijn studies. Ook voor de fijne tijden aan zee in 't Boeitje!
Lieve José! Ik zie je voor altijd aan de zijde van Papi. Ik denk met plezier terug aan de tijden in Zoersel. Bij jullie was het altijd gezellig.

Ook dank aan mijn schoonfamilie, in het bijzonder mijn schoonouders Annemie en Alex. Bedankt om in drukke tijden de zorgen voor Wannes even op jullie te nemen!

En nu ik toch over onze kleine spruit bezig ben... Wannes, je bent een fantastisch kereltje! Wat een lach van jou niet doet na een werkdag. Die slapeloze nachten in het begin verdwijnen dan in het niets.

En last but not least: Emmy. Tja, er zijn te weinig woorden voor wat jij allemaal hebt opgeofferd om het mij zo makkelijk mogelijk te maken het laatste jaar! En wat dat betekend heeft voor mij, is nog moeilijker in woorden te vatten. Vandaar voor jou de enige die wél van toepassing is:

En hier moet ik eindigen, want intussen is mijn uitroepteken versleten¡

Michiel

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List of abbreviations

A

AA-EQS	annual average environmental quality standard
AF	assessment factor
ASE	Accelerated Solvent Extractor

B

BC	black carbon
BW	body weight

C

CA	concentration addition
CCAP	Culture Collection of Algae and Protozoa
CF	correction factor

D

DMP	dimethyl phthalate
DOC	dissolved organic carbon
DOM	dissolved organic matter

E

EC	effect concentration
ECHA	European Chemicals Agency
EFSA	European Food Safety Agency
ESI	electrospray ionization
EU	European Union

G

GC	gas chromatography

H

HI	hazard index
HPLC	high-performance liquid chromatography

I

INRAM	Integrated Risk Assessment and Monitoring
ISO	International Standard Organization

L

LC	lethal concentration
LDPE	low-density polyethylene
LL-EAC	lower limit environmental assessment criteria
LOD	limit of detection
LOQ	limit of quantification

M

MB	model bias
MEC	measured environmental concentration
ML	maximum level
MOA	mode of action
MS	mass spectrometry

N

NOAEL	no observed adverse effect level
NOEC	no observed effect concentration

O

OC	organic carbon

P

PAH	polycyclic aromatic hydrocarbon
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PCC	Pearson correlation coefficient
PDMS	polydimethylsiloxane
PEC	predicted environmental concentration
PNEC	predicted no effect concentration
POC	particulate organic carbon
POM	particulate organic matter
PRC	performance reference compound
PSD	passive sampling device

Q

QSAR	quantitative structure activity relationship

R

RA	risk assessment
RCR	risk characterisation ratio
REACH	Registration, Evaluation and Authorisation and Restriction of Chemicals
RQ	risk quotient

S

SI	supporting information
SPM	suspended particulate matter
SPMD	semi-permeable membrane device
SPME	solid-phase microextraction
SSD	species sensitivity distribution
STU	sum of toxic units

T

TBT	tributyltin
TDI	total daily intake
ToF	time-of-flight
TU	toxic unit
TWA	time-weighted average

U

UK	United Kingdom

V

VP	vapour pressure

1

General introduction and conceptual framework

1.1 Introduction

All organisms are composed of chemicals – e.g. water, proteins, nucleic acids and others – and life would not be possible without numerous biochemical processes. This very simple fact illustrates the importance of chemicals for all life on our planet. As humans, we consist of these same compounds, but additionally we are the world's biggest producer and consumer of both natural and synthetic chemicals [1]. We use chemical compounds for our food, clothing and building materials as well as for a variety of activities like sanitation, recreation, decoration, etc. Many of those substances – while having contributed to increases in our life quality and expectancy – are not essential for life and often have hazardous properties. If not well managed, they have the potential to harm both man and the environment. The realization that chemicals, when released accidentally or intentionally into the environment, can adversely affect the health of wildlife is relatively recent [2]. Important early examples of adverse environmental effects of chemicals are chlorinated pesticides causing acute and chronic toxicity to terrestrial predators in the 1960s and 70s [3] and the endocrine disrupting effects of the antifoulant tributyltin (TBT) on aquatic molluscs as noted in the early 70s [4]. Combined with other human activities detrimental for the environment such as deforestation, agriculture and excessive energy consumption as well as environmental disasters such as the nuclear accident in Chernobyl or the Deepwater Horizon oil spill, these examples have increased awareness of the consequences of environmental degradation. This in turn has led to a considerable increase of fundamental and applied research in the field of risk assessment and chemical control [5].

A large number of chemical contaminants released into the environment eventually end up in seas and oceans via riverine inputs, direct discharge, atmospheric deposition and land runoff. Many marine ecosystems – including the Belgian North Sea – are under ongoing pressure from chemical pollution and other anthropogenic activities like fishing, oil and gas exploration, sand extraction, mariculture and tourism [6]. Such factors – often in combination with other anthropogenic pressures – have sometimes led to serious impacts on marine environments, causing the collapse of fish populations [7], the occurrence of so called dead zones in coastal areas [8], declines of coral reefs [9] and local biodiversity losses [10]. And yet our seas and oceans provide us with a myriad of important ecosystem services. Apart from being an important food source, the marine environment plays an important role in the global geochemical cycling of the elements necessary for living organisms (carbon, nitrogen, oxygen, etc.) and it acts upon societal wastes in various ways in order to transform, detoxify or merely sequester them [11]. As such, the protection of marine ecosystems is of vital importance, both for the environment and for human health.

While based on the above it is clear that the protection of the marine environment has multiple facets, this thesis only deals with the threat of anthropogenic chemicals. In this regard, achieving a good environmental quality depends on our ability to adequately establish the current levels of pollutants and understand their impact on ecosystem and human health. Therefore, reliable data on concentrations of chemicals and knowledge of the effects of these compounds on organisms, populations and entire ecosystems are crucial. These data are used in the process known as risk assessment to establish whether the environmental contaminants pose a risk to environmental and/or human health. This process typically consists of a comparison of contaminant levels with established safety thresholds for the compartment of concern. Exceeding the safety threshold indicates the presence of a risk. However, multiple difficulties exist in the derivation of both contaminant levels and their safety thresholds. This is mainly due to continuous variations in chemical concentrations, making it difficult to obtain reliable exposure data, and the complexity of ecosystems, complicating the reliable determination of adverse effects caused by these compounds. The latter is complicated even further due to the fact that organisms and humans are exposed to mixtures of chemicals, the composition of which is constantly changing.

Two techniques that may help reduce the abovementioned difficulties are passive sampling and passive dosing. Both techniques make use of a material – solid or liquid – that has a high affinity for either hydrophobic or hydrophilic chemicals.

1.2 Conventional risk assessment practices

Risk assessments come in many forms, depending on their scope and nature, and can be retrospective (i.e. examining chemicals already present in the environment) as well as predictive (e.g. anticipating on the future emission of a new pesticide) [5]. Typically, a risk assessment consists of an exposure assessment and an effect assessment. In the context of human health, the latter is also called a hazard assessment. In the exposure assessment, the environmental concentrations of a contaminant are determined either by direct measurement or by prediction, yielding a MEC (measured environmental concentration) or a PEC (predicted environmental concentration), respectively. In the effect assessment, the contaminant concentration considered safe for the environment (or humans) is derived, typically a PNEC (predicted no effect concentration) value (or a no observed adverse effect level (NOAEL) for humans). In the process of risk assessment, it is then investigated whether the environmental concentration exceeds the PNEC by calculating a risk characterisation ratio (RCR):

$$\mathrm{RCR} = \frac{\mathrm{MEC\ or\ PEC}}{\mathrm{PNEC}} \tag{1.1}$$

An RCR higher than 1 indicates that a risk for adverse effects is present. In the following two paragraphs, the exposure and hazard assessments will be further discussed including the associated problems, complexities and uncertainties. Predictive risk assessments – in which environmental contaminant concentrations are predicted using exposure models based on emission data – are outside the scope of this thesis and will not be further discussed.

1.2.1 Exposure assessment

Conducting an exposure assessment – through monitoring campaigns – for a retrospective risk assessment is most commonly done by collecting spot (bottle or grab) samples that are shipped to a laboratory for qualitative and/or quantitative analysis [12]. Taking samples in the marine environment is usually done on board a research vessel (Figure 1.1) for offshore areas or using rigid inflatable boats closer to shore or in harbours. Water and sediment samples are typically collected using a NISKIN or GO-FLO bottle and a Van Veen-grab, respectively (Figure 1.2), although other sampling devices also exist. Suspended particulate matter (SPM) can be collected using for example a flow-through centrifuge. Marine organisms are generally sampled using different types of nets (e.g. a beam trawl for catching benthic organisms like flatfish and shrimp).

Figure 1.1: Marine research vessel "Zeeleeuw".

The aim of monitoring contaminants in the (marine) environment can be the detection of a trend (temporal or spatial) or the non-compliance with a certain threshold or quality standard (e.g. a PNEC value) [13]. In order to make a valid

Figure 1.2: Niskin bottles mounted on a carrousel for water sampling (left) and a Van Veen-grab used for sediment sampling (right).

statement on either of these, rigorous sampling and resampling of a chemical component within a well-defined area is crucial. This is due to the high variability of the contaminant concentrations both in time and space caused by for example tidal cycles in coastal zones or flood events in riverine environments [14]. Fernandez et al. for example, studied concentrations of trace elements in water samples collected in a Spanish estuary [15]. For some of the contaminants, they found differences in metal concentrations of over an order of magnitude between samples collected at low and high tide. Moreover, at high tide the spatial distribution of the trace elements tended to be homogeneous over the estuary, while large concentration differences between sampling locations were observed at low tide. Similarly, concentrations of contaminants in sediments are susceptible to spatial variation as for example observed by Tam et al. [16]. These authors investigated metal concentrations in mangrove sediments and took samples at intervals of 5 m. In some cases they observed differences in metal concentrations up to a factor of 3 in adjacent samples. In the case of biota, the problem of spatial and temporal variability is even more complex as a large number of aquatic organisms are capable of migration.

The above clearly illustrates that contaminant concentrations measured in one grab sample of water or sediment are often not representative of local pollution. If collected concentration data are to be representative of general pollution levels in a certain area, a robust monitoring scheme with an appropriate sampling frequency and a sufficient number of sampling stations is crucial [17]. The adequacy of any sampling strategy depends heavily on the local situation (e.g. the proximity of emission sources, flushing rates of enclosed areas, local sediment dynamics, etc.). While it is thus impossible to come up with a single monitoring scheme that fits all

situations, it is important to realise that the required number of sampling stations and the sampling frequency can be substantial. Allan et al. [14] note that collecting grab samples on a monthly basis is probably not sufficient to provide a reasonable estimate of the average concentration of a chemical in a water body characterized by marked temporal and spatial variability. Given that, depending on the size of the ship and its crew, daily operating costs of oceanic research vessels generally vary between 10,000 and 30,000 $, the costs of monitoring are high [18]. Moreover, the collected samples need to be chemically analysed. For this, expensive equipment is needed and the analysis itself is labour intensive, often requiring complex extraction and clean-up techniques depending on the environmental matrix. This further adds to the costs and complexity of the exposure assessment.

1.2.2 Effect assessment

In order to establish whether the presence of a chemical in the environment can be harmful to ecosystems, information on its ecotoxicity is needed. Ecotoxicological effects are adverse changes in the state or dynamics of organisms caused by exposure to a chemical [19]. Adverse effects will first occur at the lowest levels of biological organisation (i.e. at the subcellular level) and may not produce any measurable effect on the organism or population level. This may, however, change if the exposure concentrations are high enough and are sustained for a sufficient period of time.

Information on the ecotoxicity of a substance is generally obtained through laboratory ecotoxicity testing. In the simplest form of such experiments, a specific test organism is exposed to different concentrations of a test chemical for a well defined period of time. One of the most common tests to determine the acute or short term toxicity of a substance, is the 48 hour immobility test with the freshwater flea *Daphnia magna*. In this test, groups of water fleas (also known as "daphnids") are exposed to increasing concentrations of a toxicant (Figure 1.3A). After 48h, the number of immobile daphnids (i.e. individuals that do not appear to be moving) is counted for each test concentration. The concentration of the toxicant at which 50% of the daphnids are found to be immobile, is called the EC50. This EC50 is statistically derived by fitting an appropriate model to the immobility counts (Figure 1.3B) and is one of the most common measures of acute toxicity of a substance towards the test species. To determine the long term or chronic toxicity of a substance towards organisms, other types of tests are used. For daphnids, this is typically the 21 day reproduction study in which the effect of the chemical on a number of parameters is studied. Such parameters may be the time to first brood, the number of offspring produced per female, growth and survival [20]. Similar as for the acute study, an effect concentration can be estimated, typically being an EC10 value (the concentration of the chemical causing 10% of effect) or a NOEC

(no observed effect concentration). The latter is the highest test concentration at which no effect is observed.

Of course, the main point of interest in the environmental hazard assessment of a chemical is not its specific toxicity towards a single species but rather identifying a safe level at which it does not have an effect on populations and – eventually – entire ecosystems. Of course, with increasing levels of biological organisation the number and complexity of the interactions a chemical can have, increase as well. Thus, one of the major complexities any ecotoxicologist has to deal with, is the enormous taxonomic diversity. Ehrlich and Wilson estimated that 10 to 100 million species inhabit the earth, 1.5 million of which have been taxonomically classified [21]. Of course, it is practically not possible to perform ecotoxocity tests – such as the ones described above – on a representative sample of this huge variety of species [19]. Instead, there is the need for a pragmatic approach that, based on a limited dataset, can still deliver reliable information on safe environmental levels of chemicals. To this end, ecotoxicological data are generated on a limited number of species which are selected based on their ecological function, morphological structure and their route of exposure [19]. A typical, basic dataset that is needed in current risk assessment schemes such as the one used in the European REACH[1] legislation [22], consists of acute ecotoxicity data on 3 freshwater species from different trophic levels: one aquatic plant species (typically a unicellular alga), one invertebrate species (mostly *D. magna* is used) and one fish species [23]. Of course, given the enormous biodiversity these species can hardly be representative for even a local ecosystem, which will consist of much more than just these 3 organisms. As such, in order to derive safe levels for a certain chemical (i.e. a PNEC value), assessment factors are used to address the uncertainties associated with the extrapolation from single-species ecotoxicity data to a multi-species ecosystem [23]. The PNEC is thus calculated by dividing the lowest effect concentration available (indicating the highest toxicity) by an appropriate assessment factor (AF). This AF is 1,000 when the basic dataset described above is available and may be lowered when more ecotoxicity data become available [23]. When a sufficiently large and diverse ecotoxicity dataset is available, more complex statistical methodologies may be used to derive a PNEC (i.e. the use of a species sensitivity distribution or SSD). However, this is outside the scope of this study and will not be discussed in detail.

The above mentioned procedure to derive a PNEC value (i.e. by means of an AF) may seem straightforward and simple, but many difficulties may already arise

[1] REACH (Registration, Evaluation and Authorisation and Restriction of Chemicals) is a European legislation requiring the industry to register all chemicals imported into or manufactured in Europe in quantities higher than 1 ton per year. Such a registration includes the obligation to demonstrate that the manufacturing and/or use of this chemical does not pose a risk for human health or the environment.

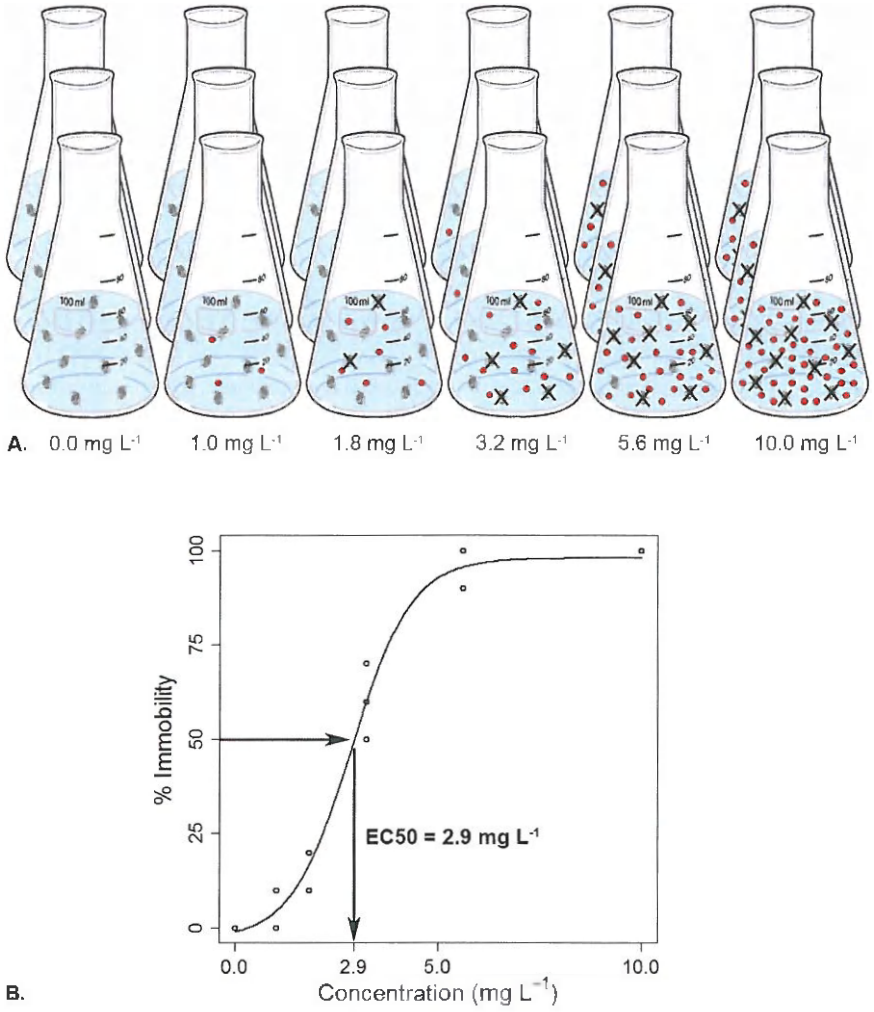

Figure 1.3: Graphical representation of a 48h Daphnia magna immobility test, a standard ecotoxicity study. A. Erlenmeyer flasks containing daphnids exposed in triplicate to increasing concentrations of a toxicant (represented by the red dots). Crossed out daphnids represent individuals being immobile after 48h. B. Graph showing the results of a 48h Daphnia magna immobility test (such as the one depicted above) with the percentage of immobile daphnids plotted against the concentration of the toxicant. The line plotted through the data shows a typical sigmoid dose-response curve fitted with a logistic model. From such a model, the EC50 value (i.e. the concentration at which 50% of the daphnids were immobile after the 48h exposure period) can be estimated, which is visually represented on the graph.

when conducting a single ecotoxicity study. Most of the difficulties are related to exposure, which is of particular importance in aquatic toxicology [19]. Indeed, while in ecotoxicity tests with terrestrial organisms (e.g. rats or birds) the toxicants are often administered directly via food or even injection, the exposure in aquatic tests is much more complicated. In the latter, the test substance is dissolved in the test medium and is taken up by the test organism through the skin and – in particular – through the gills. The concentration of the chemical in the test organism is usually unknown and therefore the toxicity is expressed as the concentration in the test medium [19]. As such, in order to determine whether or not an adverse effect will occur, careful consideration must be given to both the exposure time and concentration.

However, many chemicals possess one or more properties that complicate the maintenance of a constant exposure concentration during an ecotoxicity test. This is for example the case for chemicals that are (highly) volatile, degrade rapidly, adsorb to test vessel walls or organic matter, are (highly) bioaccumulative and/or have a low water solubility. In aquatic toxicity testing, there are generally 3 types of exposure systems: (1) static exposure systems in which the test solution is never changed or renewed, (2) the renewal or semi-static exposure system in which the test solution is periodically renewed, and (3) the flow-through system in which the test solution is constantly renewed by a continuous input of fresh medium. The static exposure system is generally only used for acute tests (< 96h) and the main advantage is its simplicity, low cost and low handling stress to the test organisms. For technical reasons, this is the only option for studies with unicellular algae. Of course, as the test solution is never renewed, the concentration of the test chemical tends to decline during the experiment. Other problems may be an unacceptable drop in oxygen levels as well as possible starvation when food may not be added (food can interfere with the bioavailability of some substances).

Most of these issues can be resolved by using a semi-static exposure system, which allows feeding and the test may be prolonged indefinitely. However, the periodical renewal of the test solution causes increased stress in the test organisms and could cause injury. Moreover, this system does not guarantee a constant exposure throughout the test and is more labour intensive than the static system. The flow-through system is much better able to maintain constant concentrations of the toxic material and also allows other important parameters to be kept constant (e.g. temperature, oxygen levels, etc.). The main disadvantage of this system is its complexity and high cost. Additionally, it can generally only be used for fish tests, as smaller organisms such as daphnids could be flushed out while other species are very sensitive to currents (e.g. copepods) [19].

1.2.3 Mixture toxicity

The previous section described the general practices to establish safe levels for individual chemicals. However, in the European Union (EU) an estimated 30,000 industrial chemicals are marketed at volumes higher than 1 tonne per year [24]. Therefore, it should be no surprise that organisms in the environment are never exposed to only a single chemical but rather to multicomponent chemical "cocktails" [25]. Indeed, studies in which the environmental concentrations of multiple chemicals are investigated, consistently demonstrate the co-occurrence of different compounds from a broad range of chemical classes in all environmental compartments (e.g. in water [26–31], in sediment [27–29, 32–34] and in organisms [27, 28, 30, 35–38]).

Within environmental risk assessment, the most important question with regards to the safety of chemical mixtures is: if all individual chemicals of a mixture are present at concentrations below their respective PNEC values (i.e. concentrations at which no adverse effects are expected), can there still be an adverse effect arising from exposure to the mixture? In order to elucidate this, a closer look at the mechanisms behind mixture toxicity is needed.

The effect that a mixture of 2 chemicals will have on an organism, depends on the mechanism through which these chemicals exert their toxicity and whether or not they will interact in one way or the other [39]. The simplest scenario is that the chemicals in the mixture work by the same mechanism or mode of action (MOA). In this case their combined toxicity will be determined by their summed concentration at the site of action, which is typically referred to as "concentration addition" [40]. A second scenario is that the chemicals work via a different mechanism and do not in any way interact. In this case their combined toxicity will be determined by the sum of the toxic responses that they cause, which is typically called "response addition" [40]. For example, if two such chemicals would individually cause 20% and 30% immobility in a 48h *D. magna* study at their respective concentrations in a mixture, this mixture will cause 50% immobility. When comparing these two scenarios, an important difference must already be emphasized. Indeed, in the case of concentration addition it is the sum of the concentrations of the individual components that determines the eventual mixture toxicity. As such, as long as a sufficient number of compounds are present in the mixture, the summed concentration may lead to an eventual mixture effect even if the chemicals are present at very low levels (i.e. below their individual effect thresholds). In the case of response addition, however, as long as the effects of all individual mixture constituents at their respective levels are zero, the sum of the responses will also be zero. Put differently, if chemicals with different toxic mechanisms (that do not interact in any way) are present in a mixture at safe levels, the mixture itself will also be safe.

Another scenario arises if chemicals in a mixture interact. Any such interaction of chemicals in a mixture may lead to effects that are either higher (synergistic) or lower (antagonistic) than would be expected based on concentration and/or response addition. A well known example of synergistic interaction is the one between P450 inhibitors and insecticides [41–44]. This interaction is even deliberately exploited in certain insecticide formulations in which piperonyl butoxide enhances the toxic effect of the active ingredient (e.g. pyrethrins) [45]. The concentration of the latter can then be reduced in the pesticide formulation, which is beneficial for the environment in the long term. It is, however, the potential existence of unforeseen synergistic interactions that caused initial mixture toxicity concerns [46]. Luckily, the presence of such interactions in environmental mixtures are found to be rare and occurring mainly when the concentrations of the chemicals of concern are high (i.e. already exceeding their individual effect thresholds) [25, 39, 40]. Therefore, it has generally been concluded that for risk assessment the focus should be on effects arising from concentration and response addition [39, 40].

While the above explains whether any adverse effects of environmental mixtures can be expected and under which circumstances, it remains to be discussed how to determine which mixtures are really of concern. It has been suggested that the concept of concentration addition can be used as a conservative estimate of the potential mixture toxicity if the MOA of the individual mixture constituents is not known. When having information on the concentrations of the individual mixture constituents, the most straightforward way to apply this concept is by calculating the sum of the toxic units (TU):

$$\sum_{i=1}^{n} TU = \sum_{i=1}^{n} \frac{C_{water,i}}{ECx_i} \tag{1.2}$$

with $C_{water,i}$ as the concentration of the i^{th} compound in the mixture and ECx_i as the concentration causing a well defined effect (e.g. an EC10 or an EC50 value). By definition, when the sum of TUs of a mixture equals 1, it is expected to cause x% of effect (e.g. 10% in the case EC10 values are used). This approach allows to make a mathematical estimation of the risk of environmental mixtures. However, this calculation only makes sense if data from the same ecotoxicity study (e.g. a 48h *D. magna* immobility study) are used, which implies these data must be available for all chemicals in the mixture. When trying to apply this to environmental mixtures containing potentially hundreds of compounds, the problem will soon arise that only for a part of them results of the same ecotoxicity study exist. Indeed, the major obstacles to reach this goal are data gaps, and such gaps in our knowledge on the MOAs and the effects of the approximately 70,000 chemicals

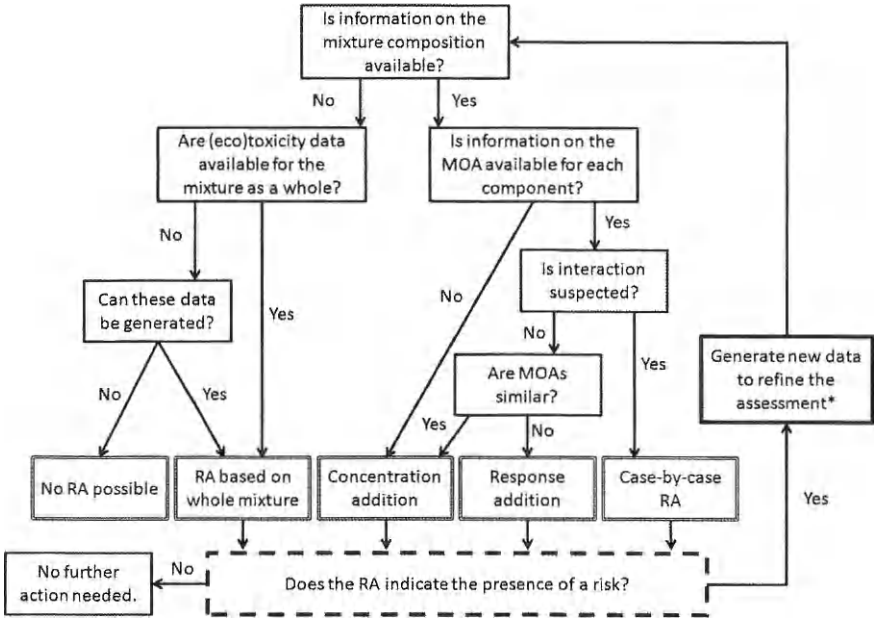

*Figure 1.4: Flowchart summarising the basic principles of tiered approaches that were developed to deal with the risks of environmental mixtures [25, 47, 48]. These approaches are typically iterative, starting with a risk assessment based on the initially available information – which is typically limited, having substantial data gaps – using conservative risk assessment methods. If risks are expected in the lower tiers, new exposure and/or effect data is generated to refine the risk assessment. MOA: mode of action, RA: risk assessment. *When no more refinement is possible, appropriate risk management measures must be taken to address the risk posed by the mixture.*

marketed in the EU are abundant [40]. As a result of this, attempts to develop a pragmatic approach for dealing with environmental mixtures have been made. All these attempts have led to similar tiered methodologies [25, 47, 48]. Figure 1.4 shows a simplified summary of these approaches, which are typically iterative, allowing for a refinement of the risk assessment by generating additional exposure and/or effect data if in the lower tier a potential mixture risk was observed. These data may be obtained via measurements and/or experiments, but may also be generated using modeling approaches (e.g. environmental fate and exposure models for calculating environmental concentrations, quantitative structure activity relationships (QSARs) for estimating ecotoxicity values) [25].

As an illustration of a lowest tier approach, Backhaus and Faust propose to use the following form of the concentration addition formula (Equation 1.2 [25]):

$$RQ_{PEC/PNEC} = \sum_{n}^{i=\hat{1}} \frac{PEC_i}{PNECi} \tag{1.3}$$

with $RQ_{PEC/PNEC}$ as the risk quotient (RQ) based on PEC and PNEC, and PEC_i and $PNEC_i$ as the PEC and PNEC of the $\hat{1}^{th}$ compound in the mixture. If the $RQ_{PEC/PNEC}$ exceeds 1, this means that the environmental quality standard (i.e. the collection of PNECs) is exceeded. Of course, as Backhaus and Faust argue [25], the data underlying the PNEC values of the different mixture constituents may be based on different groups of species. However, according to Backhaus and Faust [25] it will always give a more conservative estimation of the risk of the mixture and is therefore suitable as a worst case approach in the lowest tier.

As can be seen in Figure 1.4, there is also the possibility of using the toxicity data of the whole mixture if such data are available or can (easily) be generated. Such whole sample toxicity assessments have been found useful to help identify, diagnose and control impacts of complex contaminant mixtures on the environment [49]. Indeed, if within a monitoring framework (a selection of) samples would be subjected to such assays – which would need to include tests at different trophic levels to yield meaningful results [14] – those samples showing adverse effects can be further analysed in detail. The main advantage of this approach, compared with the mathematical methods described above (i.e. using the concentration addition model), is that with the latter the calculated toxicity is based only on the identified mixture constituents. As environmental samples are usually screened for only a selection of target chemicals, many constituents of environmental mixtures may remain unknown. Of course, if such unidentified chemicals contribute significantly to the adverse effect of the mixture, this will be observed in whole sample bioassays.

One major issue remaining with this technique relates to the problems with grab samples described in Chapter 1.2.1. As has been discussed, the concentrations of individual chemicals vary continuously and as such, so does the composition of environmental mixtures. This makes the amount of mixtures that would need to be tested virtually unlimited. In practice, this is impossible. Therefore, there is a need for an alternative sampling and exposure methodology.

1.3 Passive sampling and dosing in monitoring and hazard assessment

In Chapter 1.2, a number of issues are described with conventional monitoring and hazard assessment approaches:

(1) the concentrations of contaminants in grab samples of water, sediment and biota only offer a snapshot of the local pollution, lacking relevant information on the general pollution levels,

(2) conducting monitoring campaigns by means of oceanic research vessels and measuring contaminant concentrations in complex matrices such as sediment and biota by means of conventional analytical techniques, is expensive and labour intensive,

(3) the exposure concentrations in ecotoxicity studies are difficult to maintain throughout a test for certain chemicals (e.g. substances that are volatile, have a very low water solubility and/or tend to adsorb to the test vessel wall),

(4) while whole sample bioassays can offer valuable information for use in mixture risk assessment, performing such tests with grab samples suffers from the same shortcoming as described in (1).

Two relatively recent techniques that may help to resolve these issues at least partially, are passive sampling and passive dosing. In the following chapters, both techniques will be described as well as how they can help resolve the issues associated with conventional monitoring and risk assessment methodologies.

1.3.1 Passive sampling

Passive sampling is a methodology that has been used for monitoring air quality since the early 1970s and was extensively used by industry to monitor toxic chemicals in workplace air [50]. In the late 1980s, this technique has also been used for monitoring purposes in aqueous environments. The devices used for passive sampling are conveniently called "passive samplers" and can be defined as devices that extract chemicals from a matrix (e.g. water or sediment) in a completely passive manner. The uptake process is thus mediated by the passive diffusion of the analytes from the matrix that is sampled (where the chemical fugacity or potential is initially high) to the passive sampler (where the chemical fugacity or potential is initially low) [51]. This process continues until the chemical potential in the sampler equals the chemical potential in the sampled matrix, which is the state typically called "equilibrium". One of the most important characteristic of passive sampling is that only the freely dissolved fraction of an analyte is sampled. As this is representative of the bioavailable fraction, it is mainly responsible

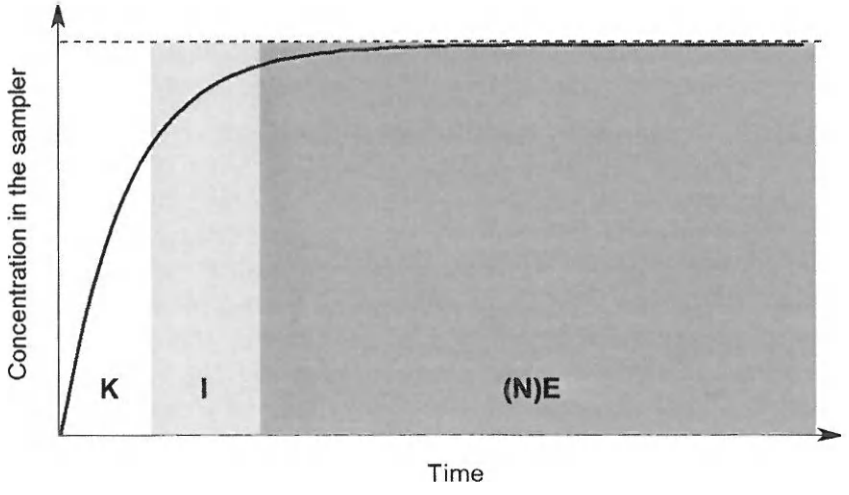

Figure 1.5: Representation of the typical uptake kinetics of a passive sampler, showing the 3 different uptake phases: the kinetic (K), intermediate (I) and (near) equilibrium ((N)E) phase. Adapted from Mayer et al. [53].

for causing toxicity in aquatic organisms. Thus, it is more meaningful than the aquatic concentrations derived by means of conventional analytical techniques as the latter generally also includes the fraction sorbed to dissolved organic matter (DOM) [52].

The uptake of chemicals from water into a passive sampler generally follows the pattern shown in Figure 1.5. In the uptake kinetics, 3 phases can be distinguished [53]: (1) the kinetic phase during which the uptake rate is constant (also known as the linear uptake phase), (2) the intermediate phase in which the uptake rate is progressively slowing down, and (3) the (near) equilibrium phase in which the uptake rate is approaching 0. The intermediate phase is not recognised in most studies (e.g. Vrana et al. [50]), possibly because it is of lesser importance for passive sampling. Indeed, two types of passive sampling principles are used: (1) equilibrium sampling, and (2) kinetic sampling.

In equilibrium sampling, the sampling duration must be long enough to allow equilibrium between the water and the passive sampler to be reached. As can be seen in Figure 1.5, once the equilibrium stage has been reached, the concentration of analyte in the sampler will remain constant over time and directly reflects the concentration of the analyte in the aqueous phase. In this case, the dissolved concentration of the analyte can be easily calculated if the sampler-water partition coefficient K_d is known. In principle, the response time of an equilibrium sampler

should be shorter than any fluctuations in the environment in order to get a good estimation of the dissolved contaminant concentration at the time of sampling. This is why equilibrium samplers typically are small devices, such as the solid-phase microextraction (SPME) fibres which were first introduced in 1990 [54].

In kinetic sampling on the other hand, the sampling period is ended while the sampler is still working in the linear uptake regime [50]. As can be deduced from Figure 1.5, depending on when the sampling process is ended within the kinetic regime, the concentration of the analyte in the sampler can differ greatly. As such, without any knowledge on how far the uptake phase has advanced, it would be impossible to determine the corresponding aqueous concentration of the analyte. To this end, knowledge on the sampling rate R_s is needed. Unfortunately, R_s is strongly affected by turbulence, temperature and biofouling [50]. In order to determine R_s for a specific sampling event, either knowledge on sampling rates under controlled laboratory conditions are combined with site-specific data on water flow and temperature, or performance reference compounds (PRCs) are used. The latter are a set of chemicals that do not occur in the environment which are spiked onto the samplers in known quantities prior to the deployment in the field. Based on the amount of PRCs that have dissipated from the sampler at the end of the sampling event, an estimation of R_s can be made [55, 56]. Despite the fact that kinetic sampling is thus more complex than equilibrium sampling, it has the advantage that it allows the calculation of so-called time-weighted average (TWA) concentrations. As the name suggests, this represents the average dissolved contaminant concentrations that occurred during the sampling period. This provides a more relevant picture of organism exposure than concentrations measured in one or a few grab samples [51].

One of the best known passive sampling devices is the semi-permeable membrane device (SPMD). SPMDs are composed of a low-density polyethylene (LDPE) lay-flat tubing filled with a high molecular weight lipid, typically triolein [50, 57]. The contaminants diffuse through the LDPE membrane into the triolein, which has a high absorption capacity for hydrophobic compounds. Passive sampling by SPMDs is one of the most mature techniques for sampling organic pollutants. Indeed, the literature on sampling methodologies with SPMDs is substantial [58] and these devices have – with varying success – also been used extensively to estimate contaminant concentrations in tissues of organisms [59].

The main disadvantage of SPMDs is their design: the preparation of these samplers is difficult and the LDPE membrane may tear during handling or sampling. Therefore, there is a trend towards the use of single-phase passive samplers. These devices consist of a single solid phase which makes their handling much more practical. Passive samplers made of polydimethylsiloxane (PDMS) – the type used in this thesis – is one example of a single-phase sampler that has a high affinity

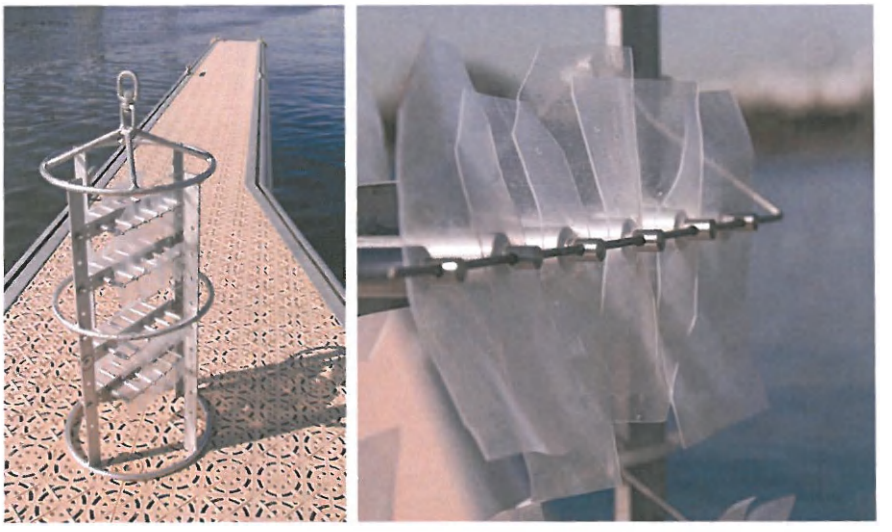

Figure 1.6: Polydimehtylsiloxane passive samplers mounted in a stainless steel cage prior to field deployment.

for hydrophobic chemicals. For environmental monitoring purposes, it is usually used in the form of thin sheets (typically with a thickness of 1 mm) and mounted in stainless steel cages (Figure 1.6). It is characterized by a relatively slow uptake phase, allowing sampling periods of several weeks. This makes it a good sampling material for determining TWA concentrations of hydrophobic organic pollutants.

1.3.2 Passive dosing

Passive dosing is a technique that involves the same type of materials as those used for passive sampling. In fact, the former relies on the exact same principles as the latter. More precisely, passive dosing is passive sampling in reverse. In passive dosing experiments, a solid phase such as PDMS is first loaded with a toxicant and is then transferred to uncontaminated test medium. The technique is mainly used for hydrophobic compounds, the concentrations of which tend to decline during ecotoxicity experiments due to several processes. Such processes may be adsorption to test vessel walls and organic matter, (bio)degradation, evaporation and bio-uptake. The solid phase effectively functions as a reservoir, replenishing any toxicant that may disappear from the dissolved phase (Figure 1.7). Passive dosing has first been applied in the late 1990s [60] and is more and more used (e.g. [61–66]).

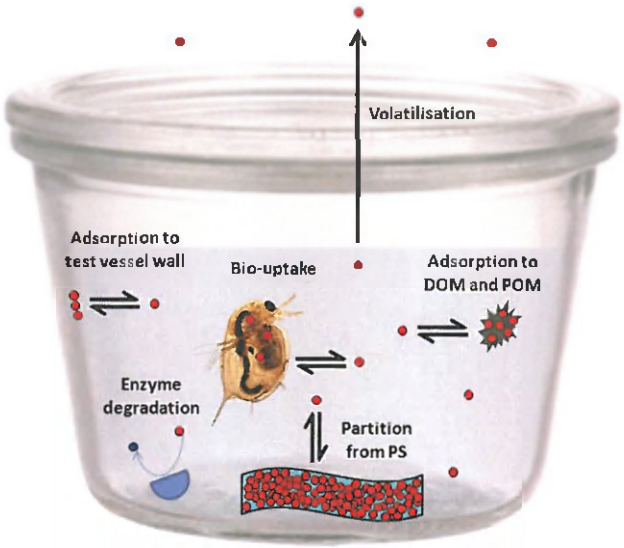

Figure 1.7: Graphical representation of a test vessel containing a solid phase loaded with a contaminant (red dots). Losses of the substance from the dissolved phase via any of the processes indicated in the figure are replenished via partitioning from the sampler. Adapted from Brown et al. [61]. DOM: dissolved organic matter, POM: particulate organic matter, PS: passive sampler.

This technique has been applied on different scales, ranging from small *in vitro* studies on bacteria using for example loaded silicone O-rings as the contaminant source [65], up to *in vivo* studies on fish using an advanced flow-through system with a cylinder containing contaminated PDMS [66]. In all passive dosing studies available today, the solid phase was loaded by spiking the contaminants via a carrier solvent. In most experiments, the test organisms were exposed to a single chemical. Up until now, only Rojo-Nieto et al. [67] have tried to recreate environmental mixtures for ecotoxicity testing. Similar as in the other studies, they loaded the PDMS phase with contaminants via spiking.

1.4 Conceptual framework of this study

This study was conducted as part of the INRAM[2] project. In this project, PDMS passive samplers play a central role as (1) a tool to monitor freely dissolved contaminant concentrations in the (marine) aquatic environment, (2) surrogates for biota in bioaccumulation assessments, and (3) a novel tool to expose organisms to environmentally relevant pollutant mixtures in laboratory ecotoxicity tests. Figure 1.8 presents the role of passive samplers in the INRAM project. The work presented in this thesis addresses these roles partly, thereby attempting to tackle a number of the issues listed in Chapter 1.3 by applying the tools described in Chapters 1.3.1 and 1.3.2. As such, the main aims of this doctoral work are to:

(1) Provide an alternative methodology to perform whole-sample bioassays – based on passive sampling and subsequent passive dosing – in which:

   a. environmentally relevant contaminant mixtures can be tested: i.e. the test concentrations will represent environmental concentrations of contaminants averaged over a longer period rather than reflect only a single moment in time (as is the case when using environmental grab samples), and

   b. the exposure concentrations will remain constant over the entire test duration.

(2) Study the potential use of freely dissolved contaminant concentrations derived from passive sampling in combination with equilibrium modelling to:

   a. determine the contaminant concentrations in other marine compartments (i.e. in whole water samples, sediment, suspended matter and biota), and

   b. use these concentration data to perform an environmental and human health risk assessment.

As the passive samplers will be used both for the exposure as the effects assessment, these devices will take up a central role in the risk assessment.

The work performed in this doctoral thesis is described in 5 main chapters. In **Chapter II**, ecotoxicity data on emerging contaminants (pharmaceuticals) are generated for a marine diatom (*Phaeodactylum tricornutum*) and subsequently used to perform a first risk assessment for this species, including the risks posed by the mixtures of pharmaceuticals present in the Belgian coastal zone.

[2]INRAM stands for Integrated Risk Assessment and Monitoring of micropollutants along the Belgian coastal zone and is a project sponsored by the Belgian Federal Science Policy Office (BELSPO). More details on this project can be found on the project website: www.vliz.be/projects/inram.

In **Chapters III and IV**, a novel technique to expose test organisms (in this case the marine diatom *P. tricornutum*) to contaminant mixtures from the field is developed and applied, respectively. In this technique, passive samplers are deployed at different locations in the Belgian coastal zone where these devices collect the local contaminant mixtures by passive diffusion. After the sampling period, the samplers are transferred into uncontaminated test medium in which the contaminants collected in the field are released. The environmental mixtures that are thus regenerated in the test medium, consist of the contaminants at concentrations averaged over the sampling period. These mixtures are then used in a growth inhibition test with *P. tricornutum*. The results of these tests are interpreted using ecotoxicity data originating from **Chapter II** as well as from scientific literature, employing current mixture risk assessment methodologies.

Chapter V explores the use of equilibrium models to calculate the concentrations of pollutants (in this case polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)) in several marine compartments (i.e. whole water, sediment, suspended particulate matter and tissue of organisms) based on freely dissolved concentrations of these compounds. The latter are derived from the passive sampling campaigns conducted during the INRAM project. The model results are compared to conventional concentration data obtained by chemical analysis of spot samples. In **Chapter VI** these models are further refined and the model outputs are used to perform and environmental and human health risk assessment of PAHs and PCBs for the Belgian coastal zone. The results are compared to the findings of conventional risk assessment methodologies.

Chapter VII reviews the results from this thesis, summarizes the conclusions and offers suggestions for further research.

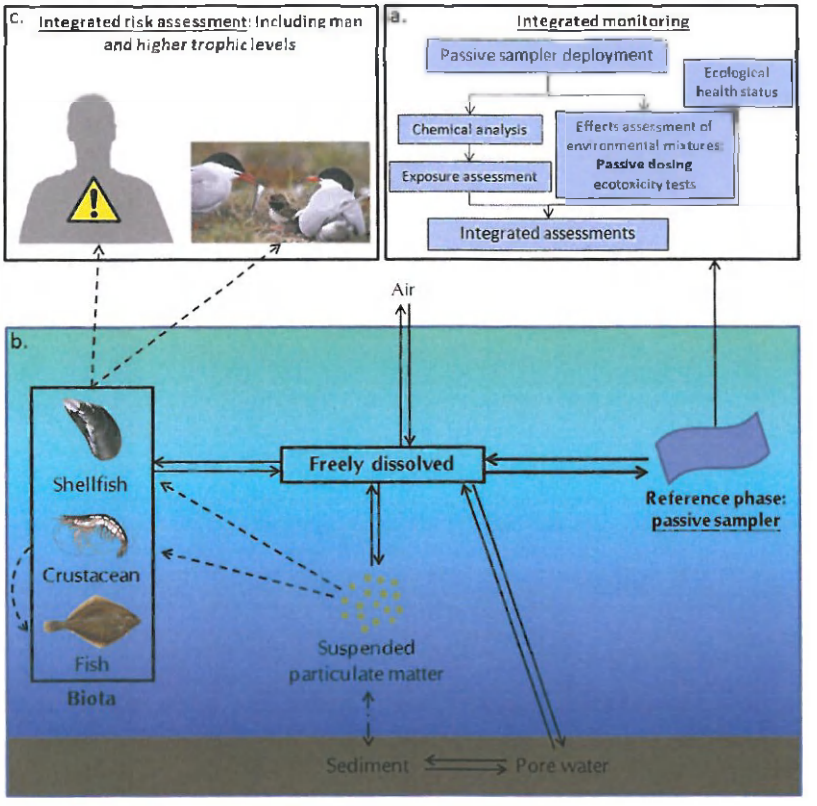

Figure 1.8: Role of passive samplers in the INRAM project. A. Test organisms are exposed to environmental mixtures absorbed by passive sampling (via the process of passive dosing) in dedicated ecotoxicity studies. Chemical analysis of the passive sampler extracts offer information of the exposure concentrations. Combined with the results of ecological monitoring studies, these data allow to assess the impact of contaminants on the health of the Belgian marine ecosystem. B. Freely dissolved contaminant concentrations derived from passive sampling are used to model the contaminant concentrations in other marine compartments (whole water, sediment, SPM and biota) via equilibrium modeling. C. The data generated on contaminant concentrations in seafood are used to conduct an integrated risk assessment for humans and higher trophic levels.

2

Emerging contaminants in Belgian marine waters: single toxicant and mixture risks of pharmaceuticals.

Redrafted from:
Claessens, M., Vanhaecke, L., Wille, K., Janssen, C.R. Emerging contaminants in Belgian marine waters: *single toxicant and mixture risks of pharmaceuticals*. Marine Pollution Bulletin, in press.

Abstract

Knowledge on the effects of pharmaceuticals on aquatic marine ecosystems is limited. The aim of this study was therefore to establish the effect thresholds of pharmaceutical compounds occurring in the Belgian marine environment for the marine diatom *Phaeodactylum tricornutum*, and subsequently perform an environmental risk assessment for these substances. Additionally, a screening-level risk assessment was performed for the pharmaceutical mixtures.

No immediate risks for acute toxic effects of these compounds on *P. tricornutum* were apparent at the concentrations observed in the Belgian marine environment. In two Belgian coastal harbours however, a potential chronic risk was observed for the β-blocker propranolol. No additional risks arising from the exposure to mixtures of pharmaceuticals present in the sampling area could be detected. However, as risk characterization ratios for mixtures of up to 0.5 were observed, mixture effects could emerge if more compounds would be taken into account.

2.1 Introduction

The occurrence of pharmaceutical compounds in the aquatic environment has received increasing attention in recent years as concerns have risen about their environmental persistence and biological activity [68]. Indeed, drug residues have been shown to occur in many freshwater (as reviewed in for example [69]) and marine ecosystems [70, 71]. These compounds end up in the environment mainly through municipal wastewater, but also due to disposal of unused medicines [72], wastewater from drug manufacturers and hospitals and landfill leachates [73]. Moreover, many of these compounds are not readily degraded in sewage treatment plants [68]. Pharmaceuticals occurring in the environment include antibiotics, painkillers, lipid regulators, β-blockers and neuroactive compounds [69].

In the freshwater environment, pharmaceuticals are generally detected at concentrations in the ng L^{-1} to μg L^{-1} range. Much higher concentrations (up to 31 mg L^{-1}) have been found in for example discharges of drug manufacturing facilities [74]. In the marine environment, reported concentrations are generally in the low ng L^{-1} range. Thomas and Hilton [75] reported concentrations up to 0.928 μg L^{-1} of the analgesic ibuprofen, and up to 0.57 μg L^{-1} of the antibiotic trimethoprim in UK estuaries. Wille et al. [71, 76] studied the occurrence of 13 pharmaceutical compounds in the Belgian coastal zone and reported concentrations of salicylic acid up to 0.855 μg L^{-1} within a Belgian coastal harbour, and up to 0.660 μg L^{-1} at open sea stations close to the shore. This compound was still detected at sampling stations located roughly 20 km off shore, at concentrations up to 0.237 μg L^{-1} and was also found in the bivalve *Mytilus edulis* at levels up to 490 ng g^{-1} dry weight. The neuroactive compound carbamazepine occurred at concentrations up to 12 ng L^{-1} at roughly 10 km off shore and was detected regularly in *M. edulis*. The remaining pharmaceuticals were only detected in the coastal harbours with a single occurrence of the β-blocker propranolol (at 1 ng L^{-1}) and the lipid regulator bezafibrate (at 8 ng L^{-1}) close to the shoreline. Propranolol was sporadically detected in *M. edulis* at levels up to 52 ng g^{-1} dry weight.

The above illustrates that contamination of the aquatic environment by pharmaceutical compounds is certainly not limited to freshwater ecosystems. Despite this, little is known about the risks these substances pose to the marine environment. Therefore, the objective of this study was to examine the toxicity of pharmaceuticals occurring in the Belgian marine environment (as studied by Wille et al. [71]) to a marine species – the diatom *Phaeodactylum tricornutum*, a standard test species [77] indigenous to the North Sea – and subsequently perform an environmental risk assessment for this environment, including the potential risks arising from mixture toxicity of the detected compounds.

Table 2.1: Physico-chemical properties of the target compounds. NSAID: non-steroidal anti-inflammatory drug. References: [78–84].

Compound	Type	log K_{ow}	Solubility in water at 20-25°C (mg L^{-1})
Salicylic acid	NSAID	2.26	2240
Paracetamol	Analgesic	0.46	12780
Carbamazepine	Neuroactive compound	2.45	112
Atenolol	β-blocker	0.16	13300
Propranolol	β-blocker	3.48	61.7
Bezafibrate	Lipid regulator	3.85	0.355
Trimethoprim	Antibiotic	0.91	400

2.2 Methodology

2.2.1 Chemicals

In total, seven pharmaceutical compounds were used for ecotoxicity testing (Table 2.1). Salicylic acid ($\geq$ 99%), paracetamol ($\geq$ 99%), carbamazepine ($>$ 90%), atenolol ($\geq$ 98%), propranolol ($\geq$ 99%), bezafibrate ($\geq$ 98%) and trimethoprim ($\geq$ 98%) were all purchased from Sigma-Aldrich (St-Louis, MO, USA).

2.2.2 Toxicity testing

The marine diatom *Phaeodactylum tricornutum* Bohlin was obtained from the Culture Collection of Algae and Protozoa (CCAP 1052/1A, Oban, United Kingdom). A subculture was maintained in the laboratory in growth medium prepared as described in the ISO 10253 standard [77]. Three days prior to the start of a growth inhibition test, a pre-culture was prepared by adding algal stock culture to fresh growth medium to obtain a cell density between 2,000 and 10,000 cells/mL. The preculture was allowed to grow on a rotary shaker at 20 $\pm$ 1 °C under continuous illumination.

Stock solutions were prepared by dissolving the pharmaceutical compounds in growth medium with the aid of ultrasonication where necessary. For each pharmaceutical compound, a series of five different test concentrations was prepared in 200 mL of growth medium by adding the correct amount of stock solution. The test solutions (including a 200 mL control medium) were allowed to equilibrate overnight at 20 °C in the dark. Subsequently, each solution was divided in 50 mL portions and transferred to a 100 mL conical flask. Three flasks were inoculated with 10,000 cells/mL of the three day old culture and one was used for a background correction. All flasks were incubated at 20 $\pm$ 1 °C under continuous white light (6,000–10,000 lx) and were shaken manually three times a day. The algal cell density was measured after 24, 48 and 72 hours using an electronic particle

counter (Coulter Counter model DN, Harpenden, Herts, UK). The temperature and pH of the test medium were measured daily.

2.2.3 Chemical analysis

Test concentrations were measured using the method by Wille et al. [71]. Briefly, samples of the test concentrations were diluted and subsequently brought to a pH 6-8. Isobutcar 61 was added to each sample as an internal standard. Solid-phase extraction of the samples was performed using Chromabond HR-X cartridges (6 mL, 200 mg, Macherey-Nagel, Düren, Germany) followed by elution using 5 mL acetone and two times 5 mL methanol. Extracts were dried using nitrogen and the residues redissolved in acetonitrile/0.02 M formic acid (50/50). Analysis was carried out using high-performance liquid chromatography (HPLC). The equipment consisted of an 1100 series quaternary gradient pump and autosampler (Hewlett Packard, Palo Alto, CA, USA) and a Nucleodur® C18 Isis HPLC column (5-μm particle size, 250 mm 4.0 mm, Macherey-Nagel, Düren, Germany). Analytes were detected with an LCQ DECA ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface (Thermo Finnigan, San Jose, CA, USA). Further details can be found in Wille et al. [71].

2.2.4 Data analysis

To estimate the EC50 and EC10 (the concentrations inducing a growth inhibition of 50% and 10%, respectively), the average specific growth rate μ was calculated for each test culture using Equation 2.1 [77]:

$$\mu = \frac{lnN_L - lnN_0}{t_L - t_0} \tag{2.1}$$

with t_0 as the time of the test start, t_L as the time of test termination (72h), N_0 as the nominal initial cell density and N_L as the measured cell density at time t_L. Subsequently a logistic response model was fitted to the concentration–response data [85]:

$$\mu = \frac{1}{1 + \left(\frac{x}{exp(a)}\right)^{ln(1/9)/(a-b)}} \tag{2.2}$$

with x as the exposure concentration, a as $\ln(EC_{50})$ and b as $\ln(EC_{10})$. For parameter estimation and calculation of the 95% confidence limits, the Levenberg–Marquardt method was used [86, 87]. All statistics were performed using the Statistica® software program (Statsoft, Tulsa, OK, USA).

2.2.5 Environmental risk assessment

The ecotoxicity data from this study were combined with literature data and subsequently used to calculate predicted no effect concentrations (PNECs) for the marine environment. To this end, an appropriate assessment factor was applied to the lowest available acute or chronic toxicity value following the rules described in the most recent guidelines relating to the European REACH legislation [22, 88]. The measured environmental concentrations (MECs) used for the risk assessment were taken from Wille et al. [71] and are summarized in Table 2.2. In this study – which was also a part of the INRAM project – water samples were collected 4 times over a time span of three years (2007–2009) in coastal harbours, off-shore locations along the Belgian coastal zone and locations on the Scheldt River (see Figure 2.1) and subsequently analysed for the presence of a set of 13 pharmaceuticals[1]. Paracetamol was also detected at the sampling stations used in this study, but the concentrations could not be quantified due to technical difficulties (unpublished data). Whenever a pharmaceutical could not be detected, the MEC was set at half the limit of quantification (LOQ). In such a case, often half the limit of detection (LOD) is used. However, as [71] did not report LODs, we used the reported LOQs. This was not considered a problem, since using the LOQ instead of the LOD makes the risk assessment more conservative (as LOQ > LOD). Hence, there was no danger of underestimating the risk. Based on the PNEC and PEC values, the risk characterization ratio (RCR) was calculated as:

$$RCR = \frac{MEC}{PNEC} \tag{2.3}$$

An RCR higher or equal to unity indicates that the ecological risks associated with the respective chemical are not adequately controlled [89].

Additionally, a screening level assessment of the risk posed by the pharmaceutical mixtures was performed using the stepwise approach proposed by Backhaus and Faust [25]. These authors propose to use the concept of concentration addition (CA) as a precautious first step in a mixture risk assessment as it generally provides the more conservative risk estimate (as compared to the concept of independent action). The risk quotient based on CA (RQ_{STU}) is calculated as:

$$\begin{aligned} RQ_{STU} &= max\left(STU_{algae}, STU_{daphnid}, STU_{fish}\right) \times AF \\ &= max\left(\sum_{i=1}^{n}\frac{MEC_i}{EC50_{i,algae}}, \sum_{i=1}^{n}\frac{MEC_i}{EC50_{i,daphnid}}, \sum_{i=1}^{n}\frac{MEC_i}{EC50_{i,fish}}\right) \times AF \end{aligned} \tag{2.4}$$

[1] Wille et al. [71] determined the environmental concentrations of the pharmaceuticals included in this work (see Table 2.1) with the exception of paracetamol. In addition, 7 other pharmaceutical compounds were targeted: mefenamic acid, ketoprofen, diclofenac, clofibric acid, chloramphenicol, ofloxacin and sulfamethoxazole. With the exception of sulfamethoxazole, none of these additional pharmaceuticals were detected.

with STU as the sum of toxic units for the respective trophic level or organism group and AF as the assessment factor. As can be seen from the formula, it is a calculation in two steps in which the STU of the most sensitive trophic level – i.e. the trophic level exhibiting the highest STU – (step 1) is used to calculate the final RQ_{STU} (step 2). AF was set at 10,000 for the marine sampling points [88].

Table 2.2: Ranges of the pharmaceutical concentrations (ng L^{-1}) measured along the Belgian coastal zone as adapted from Wille et al. [71]. SAL: salicylic acid; CAR: carbamazepine; ATE: atenolol; PRO: propranolol; BEZ: bezafibrate; TRI: trimethoprim; ND: not detected.

Station	SAL	CAR	ATE	PRO	BEZ	TRI
W01	102–660	11–19	ND	ND–1	ND–8	ND
W02	26–412	ND–14	ND	ND	ND	ND
W03	ND–106	ND–4	ND	ND	ND	ND
W04	65–227	7–12	ND	ND	ND	ND
W05	18-237	ND	ND	ND	ND	ND
W06	ND–60	ND	ND	ND	ND	ND
NP1	44–306	19–68	ND	ND–12	ND	ND
NP2	ND–94	7–54	ND	ND–12	ND	ND–17
NP3	11–177	ND–37	ND	ND–7	ND	ND
OO1	203–598	21–31	ND	ND–5	ND–5	ND
OO2	74–855	19–119	ND–88	6–24	7–18	ND–29
OO3	43–374	32–36	ND	3–11	7–12	ND–13
OO4	ND–161	16–36	ND–80	ND–12	6–11	ND
ZB1	16–136	10–30	ND	ND–3	ND	ND
ZB2	87–312	10–25	ND	ND–3	ND	ND
ZB3	80–310	11–23	ND	ND–4	ND	ND
ZB4	ND–197	11–24	ND	ND–3	ND	ND
S01	51–307	5–27	ND	ND–3	ND–6	ND
S22	71–372	129–321	ND–293	10–22	ND–16	ND

Backhaus and Faust [25] also propose the use of $RQ_{MEC/PNEC}$, which is based on the RCR of the individual mixture components and is calculated as:

$$RQ_{MEC/PNEC} = \sum_{i=1}^{n} \frac{MEC_i}{PNEC_i} = \sum_{i=1}^{n} RCR_i \tag{2.5}$$

with RCR_i as the RCR of the i^{th} of n pharmaceuticals in the mixture. While some discourage the use of $RQ_{MEC/PNEC}$ [90], it is more conservative and less dependent on the availability of a full ecotoxicity dataset than RQ_{STU} and therefore it serves well as a first screening [25]. Therefore, in this study $RQ_{MEC/PNEC}$ was calculated first for each pharmaceutical mixture. For each case in which $RQ_{MEC/PNEC}$ exceeded unity, RQ_{STU} was calculated as well.

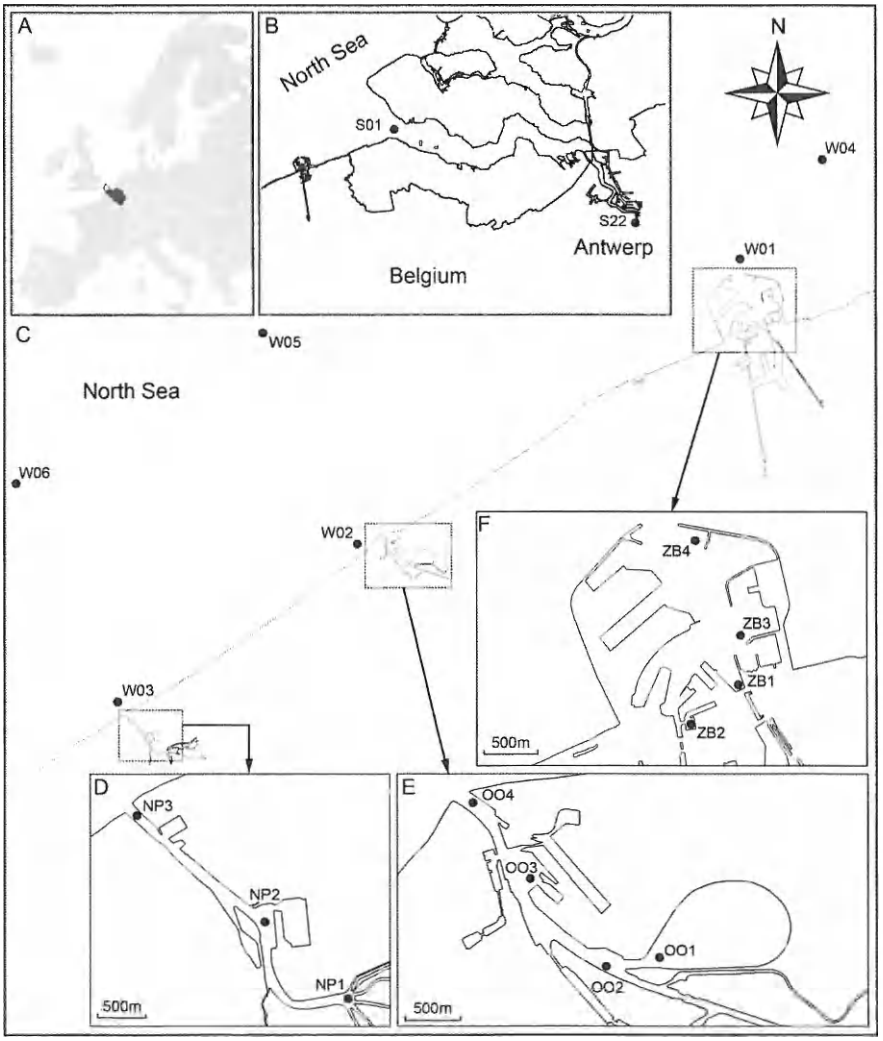

Figure 2.1: Overview of the sampling stations in the Belgian coastal zone (adapted from Wille et al. [71]). A: overview map showing the location of Belgium in Europe; B: map showing the additional sampling stations on the Scheldt River (S01 and S22); C: overview of the Belgian coast depicting the six offshore stations (W01–W06); D: detail of Nieuwpoort harbour with three sampling stations (NP1–NP3); E: detail of Oostende harbour with four sampling stations (OO1–OO4); F: detail of Zeebrugge harbour with four sampling stations (ZB1–ZB4).

2.3 Results and Discussion

2.3.1 Acute toxicity to *P. tricornutum*

Table 2.3 presents the acute toxicity of the target compounds to the marine diatom *P. tricornutum*. For bezafibrate, no effect was observed up to its limit of solubility and hence no effect concentration could be derived.

Table 2.3: Effect concentrations of the pharmaceutical compounds obtained with the 72h growth inhibition test with P. tricornutum (95% confidence limits are given between parentheses). WS: water solubility.

Substance	EC50 (mg L^{-1})	EC10 (mg L^{-1})
Salicylic acid	255.5 (242.2-269.6)	96.7 (84.9-110.2)
Paracetamol	265.8 (239.4-295.1)	93.4 (72.1-121)
Carbamazepine	62.5 (58.8-66.6)	42.2 (38.4-46.4)
Atenolol	311.9 (262.4-370.7)	6.9 (3.3-14.4)
Propranolol	0.288 (0.252-0.329)	0.09 (0.066-0.124)
Bezafibrate	>WS	>WS
Trimethoprim	5.1 (4.7-5.5)	2.4 (2-2.9)

The β-blocker propranolol and the antibiotic trimethoprim were the most toxic substances for the test organism with 72h EC_{50} values of 0.288 and 5.1 mg L^{-1}, respectively. Moreover, *P. tricornutum* seemed to be more sensitive to these substances than other (phytoplankton) species (see Figure 2.2), even though the difference is relatively small. For propranolol, this was also observed for the marine diatom *Cyclotella meneghiniana* for which an EC_{50} value of 0.244 mg L^{-1} was reported in a 96h growth inhibition test [91]. *P. tricornutum* was much less sensitive to the other tested β-blocker atenolol and showed only an average sensitivity compared to other phytoplankton (see Figure 2.2). The fact that zooplankton generally also exhibit a greater sensitivity towards propranolol compared to atenolol, has been attributed to the strong membrane stabilizing properties of the former [68]. As such, (marine) diatoms may be more sensitive than green algae to adverse effects on membrane stability, but this is at this point speculative. For trimethoprim, no data for diatoms could be found in literature, but green algae in general seem to be sensitive to antibiotics as well. Indeed, in one study the green alga *Pseudokirchneriella subcapitata* exhibited EC50 values for antibiotics between 0.002 mg L^{-1} (clarithromycin) and 0.52 mg L^{-1} (ofloxacin [92]). Yang et al. [93] reported an EC50 of 40 mg L^{-1} for trimethoprim for the same species. If *P. tricornutum* would display a similar sensitivity pattern towards antibiotics, this would imply that antibiotics other than trimethoprim (e.g. clarithromycin) could be highly toxic to this marine diatom. Further studies are warranted to confirm this hypothesis.

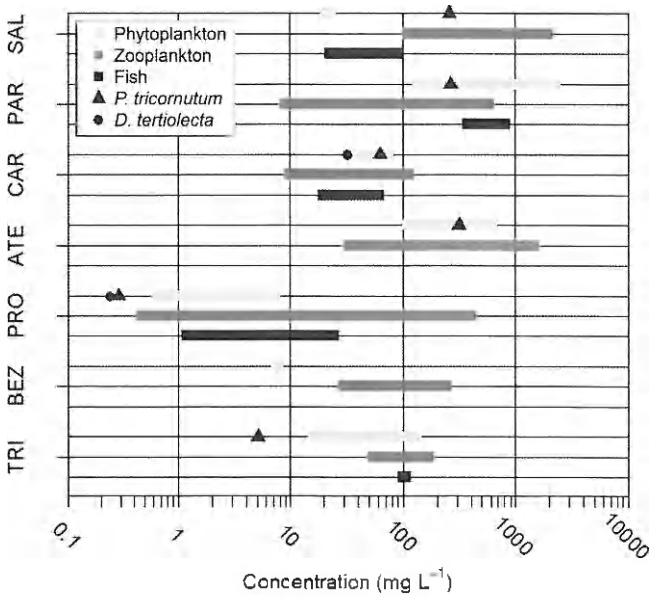

Figure 2.2: Acute toxicity of the target compounds to aquatic organisms. The bars represent ranges of toxicity data from different organisms and/or experiments. Data of the marine diatoms Phaeodactylum tricornutum and Dunaliella tertiolecta are from this study and from [91], respectively. Other references: [91, 93–127]. A full list of the literature ecotoxicity data can be found in Table A.1. SAL: salicylic acid; PAR: paracetamol; CAR: carbamazepine; ATE: atenolol; PRO: propranolol; BEZ: bezafibrate; TRI: trimethoprim.

All other tested pharmaceuticals showed moderate (carbamazepine) to low (salicylic acid, paracetamol and atenolol) acute toxicity towards *P. tricornutum*. In general, the same observation has been made for (mainly freshwater) organisms of other trophic levels. Indeed, Fent [68] summarized that the majority of the most studied pharmaceuticals have E/LC50 values above 1 mg L^{-1} and about 38% exhibit E/LC50 values above 100 mg L^{-1}. As noted above, antibiotics in general and the β-blocker propranolol in specific form exceptions.

2.3.2 Environmental risk assessment

When comparing the MECs (Table 2.2) to the ecotoxicity data generated for *P. tricornutum* in this study, no acute toxicity is expected at any of the sampling stations for the test species. Indeed, as the highest measured concentrations of the pharmaceuticals are between roughly 130,000 (for carbamazepine) and 4,000 (for propranolol) times lower than their respective EC10 values, any acute toxic effects towards the test species are highly unlikely. This finding is similar to the con-

clusion by Fent [68] who stated that acute toxicity of pharmaceuticals to aquatic organisms in general, is unlikely to occur at the measured concentrations.

Table 2.4 presents the PNEC values for the marine environment as derived from the data of this study and data from literature. For sampling station S22 (located far upstream the Scheldt river; see Figure 2.1) separate PNEC values for freshwater were derived using a lower assessment factor (AF). This AF was generally a factor 10 lower than the AF used for the marine aquatic environment [88]. The maximum RCRs determined for the different sampling periods are presented in Table 2.5. The RCRs indicated a potential ecological risk from chronic exposure to propranolol at five sampling stations: two in the harbour of Nieuwpoort, three in the harbour of Oostende. At stations NP1, NP2, OO3 and OO4 the RCR of propranolol exceeded unity only once over the four sampling periods. At station OO2 this occurred three times (in May 2007, April 2008 and June 2009). For all other pharmaceuticals, no potential chronic risk could be identified. Similar risk assessments are scarce and have been performed exclusively for the freshwater environment. Cleuvers [107] for example, studied the risk of three β-blockers (including atenolol and propranolol) in freshwater environments, of which only propranolol exhibited an RCR close to 1. Halling-Sørensen [102] studied the environmental risks of three antibiotics and came to a similar freshwater RCR for trimethoprim (i.e. $9.4\ 10^{-3}$). Han et al. [114] performed an environmental risk assessment for seven pharmaceuticals (including salicylic acid, paracetamol and carbamazepine) in the effluent of wastewater treatment plants and did not identify a risk. And finally, in a case study involving atenolol in the EU [126], a maximum RCR of 0.003 was observed in freshwater under a worst case scenario. This is similar to the RCR values for atenolol observed in this study (see Table 2.5).

Table 2.4: PNEC values of the test compounds for the marine environment derived from this study and literature review. The ecotoxicity values used for the PNEC derivation are presented. AF: assessment factor (selected according to ECHA [88]), PNEC: predicted no effect concentration.

Pharmaceutical	Concentration (mg L^{-1})	Exposure duration	Assessment endpoint	Organism	Species	Reference	AF	PNEC (ng L^{-1})
Salicylic acid	5.6	21 d	Reproduction, NOEC	Invertebrate	*D. longispina*	[110]	500	11,200
Paracetamol	9.2	48 h	Immobility, EC50	Invertebrate	*D. magna*	[95]	10,000	920
Carbamazepine	0.025	7 d	Reproduction, NOEC	Invertebrate	*C. dubia*	[91]	100	250
Atenolol	3.2	28 d	Growth, NOEC	Fish	*P. promelas*	[128]	100	32,000
Propranolol	0.001	27 d	Reproduction, NOEC	Invertebrate	*H. azteca*	[104]	100	10
Bezafibrate	0.023	7 d	Reproduction, NOEC	Invertebrate	*C. dubia*	[116]	1,000	23
Trimethoprim	2.4	72h	Growth, EC10	Diatom	*P. tricornutum*	This study	500	4,800

Table 2.5: Maximum values of the risk characterization ratios (RCRs) determined for the different sampling periods. Bold values emphasis stations at which the RCR was higher than 1. SAL: salicylic acid; CAR: carbamazepine; ATE: atenolol; PRO: propranolol; BEZ: bezafibrate; TRI: trimethoprim.

Station	SAL	CAR	ATE	PRO	BEZ	TRI
W01	0.059	0.076	0.001	0.100	0.348	0.001
W02	0.037	0.056	0.001	0.050	0.109	0.001
W03	0.009	0.016	0.001	0.050	0.109	0.001
W04	0.020	0.048	0.001	0.050	0.109	0.001
W05	0.021	0.010	0.001	0.050	0.109	0.001
W06	0.005	0.010	0.001	0.050	0.109	0.001
NP1	0.027	0.272	0.001	**1.200**	0.109	0.001
NP2	0.008	0.216	0.001	**1.200**	0.109	0.004
NP3	0.016	0.148	0.001	0.700	0.109	0.001
OO1	0.053	0.124	0.001	0.500	0.217	0.001
OO2	0.076	0.476	0.003	**2.400**	0.783	0.006
OO3	0.033	0.144	0.001	**1.100**	0.522	0.003
OO4	0.014	0.144	0.003	**1.200**	0.478	0.001
ZB1	0.012	0.120	0.001	0.300	0.109	0.001
ZB2	0.028	0.100	0.001	0.300	0.109	0.001
ZB3	0.028	0.092	0.001	0.400	0.109	0.001
ZB4	0.018	0.096	0.001	0.300	0.109	0.001
S01	0.027	0.108	0.001	0.300	0.261	0.001
S22	0.003	0.128	0.001	0.440	0.070	0.000

Table 2.6 presents the $RCR_{MEC/PNEC}$ values of the pharmaceutical mixtures at the different sampling stations. Overall, trimethoprim and atenolol combined contributed less than 1% and propranolol and bezafibrate combined contributed roughly 77% to the toxicity of the mixtures (see Table A.2). This was due to the high and low respective PNEC values of these two pairs of pharmaceuticals. Indeed, even at the six sampling stations at sea propranolol and bezafibrate were the two most dominant chemicals despite being mainly present at levels below their respective LOQs. Besides the seven occasions identified above for which there was already a risk caused by an individual pharmaceutical compound, three additional cases were identified posing a potential risk originating from the mixture of pharmaceuticals. For these three cases, RCR_{STU} was calculated (see Table 2.7) which no longer indicated a potential risk posed by the pharmaceutical mixtures. However, given that only six compounds were included in the mixture risk assessment, the RCR_{STU} values – which ranged from 0.33 to 0.50 – were nonetheless relatively high. Indeed, as in the studied area many more chemicals (e.g. pesticides, organotins, perfluorinated compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls) are present [129–132], it is not unlikely that a risk from

mixtures might arise when taking other compounds into account as well. Moreover, as potential interactions between chemicals in the mixture are not taken into account with the present approach [25], a more profound risk assessment of the mixtures present in this sampling zone is certainly warranted. This is enforced by the results of Ginebrada et al. [133], who calculated hazard indices (similar to RCR_{STU} of this study) for pharmaceutical mixtures occurring in a Spanish river basin. These authors found consistently higher hazard indices (HI) for algae compared to other trophic levels, with values up to 103. The antibiotic sulfamethoxazole, the lipid regulator gemfibrozil and the nonsteroidal anti-inflammatory drug ibuprofen were by far the most important contributors (out of a total of 24 pharmaceuticals) to the identified risks to algae. Of all the pharmaceuticals included in our study, only propranolol was not included in the work of Ginebrada et al. [133]. While this compound was identified as the most toxic in our study, this nonetheless illustrates that there are multiple other pharmaceuticals that can have a profound impact on the cumulative risk of these compounds. As such, more research is needed before risks of pharmaceutical mixtures to the marine aquatic environment can be confirmed or excluded. Such research should include the generation of more ecotoxicity data for marine species and studies on potential interaction between different mixture constituents.

Table 2.6: The $RCR_{MEC/PNEC}$ (as determined using Equation 2.5) of the pharmaceutical mixtures detected at the different sampling stations. Bold values emphasis stations at which the $RCR_{MEC/PNEC}$ was higher than 1. Underlined values indicate cases for which no risk originating from an individual pharmaceutical was observed.

	Sampling campaign			
Station	May 2007	Dec 2007	Apr 2008	Jun 2009
W01	0.244	0.345	0.473	0.228
W02	0.173	0.195	0.222	0.207
W03	0.175	0.180	0.182	0.171
W04	0.194	0.207	0.216	0.209
W05	0.172	0.173	0.176	0.192
W06	0.171	0.176	0.175	0.176
NP1	0.830	**1.591**	0.606	0.264
NP2	0.473	**1.537**	0.395	0.189
NP3	0.172	0.969	0.109	0.186
OO1	0.865	0.580	0.557	0.287
OO2	**3.741**	**1.015**	**2.763**	**2.304**
OO3	–	**1.572**	**1.550**	0.916
OO4	0.641	**1.511**	**1.613**	0.241
ZB1	0.259	0.279	0.541	0.202
ZB2	0.212	0.271	0.518	0.240
ZB3	0.214	0.603	0.410	0.268
ZB4	0.258	0.524	0.404	0.225
S01	0.185	0.295	0.679	0.244
S22	0.642	0.286	0.526	0.365

Table 2.7: The sum of toxic units (STU) per trophic level and RC_{STU} for the mixtures for which a potential risk was identified. Cases in which there was already a risk posed by an individual mixture constituent are not included. Bold values indicate the highest STU values, which were subsequently used for the calculation of RC_{STU} according to Equation 2.4. AF: assessment factor.

Sampling event	Algae	Daphnid	Fish	AF	RC_{STU}
OO2–Dec2007	$\mathbf{4.30{\cdot}10^{-05}}$	$1.86{\cdot}10^{-05}$	$2.17{\cdot}10^{-05}$	10000	0.43
OO3–Dec2007	$\mathbf{5.04{\cdot}10^{-05}}$	$2.51{\cdot}10^{-05}$	$2.02{\cdot}10^{-05}$	10000	0.50
OO4–Dec2007	$\mathbf{4.49{\cdot}10^{-05}}$	$2.55{\cdot}10^{-05}$	$1.47{\cdot}10^{-05}$	10000	0.45

2.4 Conclusions

Ecotoxicity data for seven pharmaceuticals were generated for the marine diatom *P. tricornutum* in a 72h growth inhibition test. The resulting data indicated no immediate risk for acute toxic effects of these compounds at the concentrations present in the Belgian marine environment. At five sampling stations in two Belgian coastal harbours, a potential chronic risk was observed for the β-blocker propranolol. No additional risks arising from the exposure to mixtures of pharmaceuticals present in the sampling area could be detected. However, as RCR_{STU} values of up to 0.5 were observed, mixture effects could emerge when more compounds are taken into account. Therefore, more studies on the potential risks of pharmaceutical mixtures for the marine environment are required. Such studies should focus on the generation of more ecotoxicity data for marine species and on potential interactions between mixture constituents.

3

Development of a passive dosing technique to assess the ecotoxicity of realistic environmental contaminant mixtures.

Redrafted from:
Claessens, M., Monteyne, E., Roose, P., Janssen, C.R. Passive sampling reversed: passive dosing as a technique to assess the ecotoxicity of realistic environmental contaminant mixtures. Aquatic Toxicology, submitted.

Abstract

Over the last 15 years, passive sampling devices have been used as a source of contaminants in (eco)toxicity tests to (1) achieve exposure to environmentally relevant contaminant mixtures or (2) establish constant exposure concentrations of hydrophobic chemicals (also known as passive dosing). In Chapters 3, a new approach combining these two types of studies is developed for use in Chapter 4. To this end, both modelling exercises and experimental studies were conducted to examine the use of PDMS sheets as a source of contaminants in ecotoxicity studies. PDMS sheets were able to generate – through passive dosing – steady concentrations of hydrophobic compounds (log $K_{ow} > 2$) during a 72h experiment. Less hydrophobic substances (log $K_{ow} < 2$) are likely to become significantly depleted in the sampler, causing a deviation from equilibrium conditions. As such, the passive dosing tool designed in this chapter is most suitable for compounds with log K_{ow} values > 2.

3.1 Introduction

Since the development of semipermeable membrane devices (SPMDs) by Huckins et al. [57, 134], passive sampling has become an important tool for the environmental monitoring of aquatic pollutants. Passive sampling methods are low-tech and cost-effective monitoring tools, allowing the determination of freely dissolved contaminant concentrations that are – depending on the used methodology – averaged over the sampling period [135]. Moreover, nonpolar passive samplers can concentrate hydrophobic compounds (typically present in the water phase at very low concentrations) up to levels that can be easily analysed with standard equipment [136]. Thus, many of the disadvantages associated with active sampling techniques can be avoided by using passive sampling methodologies. Over the past two decades, this has led to the development of a myriad of new passive sampling materials (see for example Zabiegala et al. [135] for a review).

Passive sampling materials have also been used more and more as a source of contaminants for (eco)toxicity testing in two types of experiments, the goals of which are markedly different. The first type aims to expose test organisms to environmentally relevant contaminant mixtures. In the earliest of these experiments, SPMDs – of which the extracts were spiked in the (eco)toxicological test medium (e.g. Parrot et al. [137]) or were even directly injected in the test organism [138, 139] – were the most popular, although similar experiments have been conducted with other types of passive samplers (an overview of passive dosing studies is given in Table 3.1). As these SPMDs had been previously deployed in the aquatic environment, the test organisms were thus exposed to mixtures directly collected in the field. In a number of these studies, chemical analysis was performed on the passive sampler extracts in which mostly PAHs [139–144], PCBs [139,143,144] and pesticides [139,143,145–147] were the target substances. While in some of these studies a correlation between contaminants and the observed effects was found [142, 148], an elaborate interpretation of mixture toxicity is generally lacking in this type of experiments.

In the second type of study, the main aim was to establish constant exposure concentrations during the entire duration of an (eco)toxicity experiment by partitioning of test substances in the test medium from a solid phase [60]. This approach – which is generally referred to as passive dosing – is mainly used for sparingly water-soluble chemicals, as they are difficult to dissolve in water and their aquatic concentrations tend to decline during (eco)toxicity testing due to adsorption of the substance to the test vessel walls, uptake by the test organism, volatilization and biotic and abiotic degradation reactions [60]. By placing a dominating solid phase such as polydimethylsiloxane (PDMS) sheets [149] or even Teflon stir

bars [62] loaded with test substance in the (eco)toxicological test medium, dissolved concentrations of the test substance are established as it diffuses into the medium until equilibrium has been reached. As any test substance that disappears from the test medium (via any of the aforementioned routes) is thus replenished by the solid phase, concentrations are kept constant over time [60]. All such passive dosing studies available in literature (Table 3.1), have been conducted with artificially spiked, nonpolar solid phases. Up to now, these studies have never been conducted with field deployed passive samplers.

The aim of Chapters 3 and 4 is to combine the two approaches described above by using passive samplers previously deployed in the field as a dosing device in a growth inhibition test with a marine diatom. As such, field mixtures of micropollutants collected by passive sampling are recreated in laboratory test medium by reversely using the samplers as dosing devices. The contaminant concentrations are expected to remain constant over time. In this Chapter, the suitability of polydimethylsiloxane (PDMS) sheets for this type of experiment will be explored. Such single-phase passive samplers are easier to use and lower in costs than multiphasic samplers (e.g. SPMDs) and have the possibility to be re-used [150]. PDMS was chosen as compounds generally show higher diffusion coefficients in this material compared to other materials [151]. This allows a more rapid uptake of contaminants from the (aqueous) environment as well as a faster release in the test medium during passive dosing experiments. Moreover, PDMS sheets are robust and can withstand exposure in the marine environment without protection (see Figure 1.6). A combination of model calculations and equilibrium experiments is used. Additionally, an experiment is conducted to ascertain that the effect of uncontaminated samplers on the growth of the marine diatom *P. tricornutum* is negligible.

3.2 Methodology

The passive sampling/dosing material used in this chapter as well as in Chapters 4 to 6, is polydimethylsiloxane (PDMS) sheet (AlteSil Laboratory Sheet, Altec Products Ltd, Bude, United Kingdom) with a thickness of 0.5 mm. All modelling calculations described below are made for this material.

3.2.1 Modelling

Model calculations were made in order to estimate (1) the extent to which substances of differing hydrophobicity are depleted from a contaminated solid phase of certain weight when transferred to 0.05 L of water, and (2) to make a rough worst-case calculation of the time needed to achieve chemical equilibrium.

Table 3.1: Overview of available literature on studies in which a passive sampling device was used as a source of contaminants in ecotoxicity testing.

Reference	Sampling/dosing device	Origin of contaminants[1]	Assay type[2]	Exposure[3]
[152]	SPMD	Environmental	In vitro (F,CL)	Extract spiked
[138]	SPMD	Environmental	In vivo (F)	Injection of extract
[60]	Empore^{TM} disk	Spiked	In vivo (A)	Passive dosing
[137]	SPMD	Environmental	In vitro (F,CL)	Extract spiked
[153]	SPMD	Environmental	Bacteria	Extract spiked
[139]	SPMD	Environmental	In vivo (F)	Injection of extract
			Bacteria	Extract spiked
[154]	SPMD	Environmental	In vivo (I,Aq)	Extract spiked
			In vitro (H,CL)	Extract spiked
[61]	PDMS cast in vial	Spiked	Bacteria	Passive dosing
[155]	Empore^{TM} disk	Spiked	In vivo (I,Aq)	Passive dosing
[156]	PDMS cast in vial	Spiked	In vivo (F)	Passive dosing
[157]	SPMD	Environmental	Bacteria	Extract spiked
			In vivo (I,Aq)	Extract spiked
[62]	Teflon stir bar	Spiked	In vivo (A)	Passive dosing
[158]	SPMD	Environmental	Bacteria	Extract spiked
			In vitro (F,CL)	Extract spiked
[140]	SPMD	Environmental	Bacteria	Extract spiked
			YES	Extract spiked
[159]	Biosilon beads	Spiked	In vitro (F,CL)	Cells attached to PS
[160]	SPMD	Environmental	YES	Extract spiked
[141]	Biosilon beads	Environmental	In vitro (F,CL)	Cells attached to PS
[161]	Silica gel	Spiked	In vivo (I,Aq)	Passive dosing
[142]	SPMD	Environmental	In vitro (M,CL)	Extract spiked
[162]	Chemcatcher	Environmental	Bacteria	Extract spiked
[163]	PDMS cast in vial	Spiked	In vivo (I,S)	Passive dosing
[164]	Silicone rods	Spiked	In vivo (A)	Passive dosing
[63]	Silicone rods	Spiked	In vivo (A)	Passive dosing
[165]	PDMS sheets	Spiked	In vitro	Passive dosing
[148]	POCIS	Environmental	YES	Extract spiked
			In vivo (A)	Extract spiked
[145]	Empore^{TM} disk	Environmental	Bacteria	Extract spiked
			In vivo (C)	Extract spiked
			In vivo (I, Aq)	Extract spiked
[143]	Lipid-free tubing	Environmental	In vivo (F)	Extract spiked
[149]	PDMS sheets	Spiked	In vitro (F,CL)	Passive dosing
[166]	PDMS cast in vial	Spiked	In vivo (I, Aq)	Passive dosing
[167]	Silicone O-rings	Spiked	In vitro (H,CL)	Passive dosing
[64]	PDMS sheets	Spiked	In vitro (M,CL)	Passive dosing
[65]	Silicone O-rings	Spiked	Bacteria	Passive dosing
[168]	PDMS cast in vial	Spiked	In vivo (I,S)	Passive dosing
[146]	POCIS	Environmental	In vivo (NBF)	Extract spiked
[66]	PDMS tubes	Spiked	In vivo (F)	Passive dosing
[144]	Silicone rubber	Environmental	In vitro (F,CL)	Extract spiked
[147]	POCIS	Environmental	In vivo (NBF)	Extract spiked
[67]	PDMS cast in vial	Spiked	In vivo (I,Aq)	Passive dosing
[169]	Silicone O-rings	Spiked	Bacteria	Passive dosing
[170]	PDMS cast in vial	Spiked	In vivo (I,S)	Passive dosing
[171]	PDMS cast in vial	Spiked	In vivo (I, Aq)	Passive dosing

[1] 'Environmental' indicates the contaminants were collected by deployment of the passive samplers in a contaminated aquatic environment, 'Spiked' indicates the contaminants were spiked on the sampler/dosing device in the laboratory.

[2] F: fish, CL: cell line, A: algae, I: invertebrate, Aq: aquatic, H: human, M: mammal, S: soil, C: coral, NBF: natural biofilm

[3] 'Extract spiked' indicates the test medium was spiked with passive sampler extract (typically a solvent containing the contaminants absorbed by the sampler)

3.2.1.1 Depletion

In order to calculate the percentage of depletion of substances from the solid phase, the sorption capacity of the solid phase (V_s, expressed as an equivalent of water volume in L) was calculated as:

$$V_s = Kd \cdot m_s \tag{3.1}$$

with Kd as the solid phase-water partition coefficient (L kg^{-1}) and m_s as the weight of the solid phase (kg) introduced in the test medium. For the latter, different values between 0.001 and 0.01 kg were used.

The percentage of depletion of the substance in the solid phase (D_s) was then calculated as follows:

$$D_s = \frac{V_w}{V_w + V_s} \cdot 100 \tag{3.2}$$

with V_w as the volume of the test medium (0.05 L).

3.2.1.2 Achieving equilibrium

In this section, an attempt was made to make a rough worst-case calculation of the time needed to achieve equilibrium. This exercise was done for the four test substances (naphthalene, acenaphthene, fluorene and phenanthrene) and for a polychlorinated biphenyl (PCB180) and a polybrominated diphenyl ether (PBDE209). As PCB180 and PBDE209 are among the most hydrophobic chemicals included in the study, the time needed to reach equilibrium was expected to be the longest for these substances. As such they were included to represent the worst case scenario. In order to model the release of chemicals from the solid phase, a one compartment model was used [172]:

$$C_w = \frac{C_s}{Kd} \cdot \left(1 - e^{-k_e \cdot t}\right) \tag{3.3}$$

with C_w as the compound concentration in water (µg L^{-1}), C_s as the compound concentration on the solid phase (µg kg^{-1}), Kd as the solid phase-water partition coefficient (L kg^{-1}), k_e as the system- and chemical-specific elimination rate constant (h^{-1}) and t as the time (h). The elimination rate constant k_e was calculated as [172]:

$$k_e = \frac{D_w \cdot A}{\delta_w \cdot V_w} \tag{3.4}$$

with D_w as the diffusion coefficient of the compound in water (cm^2 s^{-1}), A as the surface area of the solid phase (68 cm^2, the surface area of a sampler of 2.15

g as used in one of the subsequent equilibrium experiments), δ_w as the aqueous boundary layer thickness (µm) and V_w as the aqueous phase volume (0.05 L). The aqueous boundary layer thickness was calculated as [173]:

$$\delta_w = 0.526 \cdot r \cdot \sqrt[5]{\frac{\nu}{r^2 \cdot 2 \cdot \pi \cdot n}} \tag{3.5}$$

with r as the radius of the rotational movement of the rotary shaker (approximately 1 cm), ν as the kinematic viscosity of water at 20°C (1.002 10^{-6} m^2 s^{-1} [174]) and n as the shaking frequency (approximately 3 rotations per second).

The substance specific diffusion coefficients were estimated according to the approach proposed by Abraham and McGowan [175]:

$$D_w = \frac{13.26 \cdot 10^{-5}}{\nu^{1.14} \cdot V_i^{0.589}} \tag{3.6}$$

with V_i as the molar volume of the chemical, which can be estimated by summing up the atomic volumes (cm^3 mol^{-1}, Table 3.2) of all elements in the compound and subtracting 6.56 cm^3 mol^{-1} per bond (single, double or triple bonds are treated alike). For example, the total molar volume of fluorene (Figure 3.1) would be calculated as follows:

$$V_{i,fluorene} = (13)(16.35) + (10)(8.71) - (25)(6.56) = 135.65 cm^3 mol^{-1} \tag{3.7}$$

Table 3.2: Characteristic atomic volumes (from Abraham and McGowan [175])

Atom	V_{at} (cm^3 mol^{-1})
C	16.35
H	8.71
O	12.43
Cl	20.95
Br	26.21

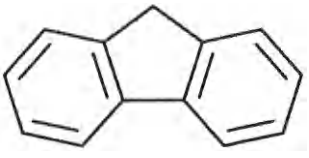

Figure 3.1: Structure of fluorene.

The time needed to reach 95% of the equilibrium (T95) was calculated as follows [172]:

$$T95 = \frac{2.99}{k_e} \tag{3.8}$$

3.2.2 Equilibrium experiments

To ensure the passive dosing setup in this study adequately maintains contaminant concentrations as observed in other studies (e.g. Kramer et al. [149]), two equilibrium experiments were conducted. All compounds in this section were purchased at Sigma-Aldrich (Saint Louis, MO, USA) and had a purity of at least 98%. The octanol-water partitioning coefficient log K_{ow}, the PDMS-water partitioning coefficient log K_d and the vapour pressure (VP) of all PAHs used in these experiments are presented in Table 3.3. In the first experiment, PDMS samplers (1.75g each) were spiked with fluorene and fluoranthene in order to reach an aqueous concentration of 2 μg L^{-1}. Spiking of the samplers was done in batch according to the methodology of Booij et al. [55] using an 80/20 (v/v) methanol-water mixture. After spiking, the samplers were transferred to 100mL conical flasks containing 50mL of algal growth medium [77] and placed on a rotary shaker for 24 hours. Flasks were removed at 1, 9, 17, and 24 hours and the concentration of fluorene and fluoranthene were measured according to the method described by Monteyne et al. [176].

In the second experiment, PDMS samplers (2.15g each) were spiked with naphthalene, acenaphthene, fluorene and phenanthrene using the same methodology as in the first experiment and subsequently transferred to 100mL conical flasks containing 50mL of algal growth medium [77]. After a 24 hour equilibration time on a rotary shaker, *P. tricornutum* was added to each flask at a cell concentration of 10,000 cells mL^{-1}. All flasks were then incubated at 20 ± 1 °C under continuous white light (6,000–10,000 lx) and were shaken manually three times a day. The concentrations of the 4 PAHs were monitored for 72 hours starting at the addition of *P. tricornutum*.

3.2.3 Effects of light attenuation by passive samplers on growth of P. tricornutum

As the passive dosing setup will be used in a 72h algal growth inhibition assay with *P. tricornutum* (see Chapter 4), the effect of light attenuation by the PDMS passive samplers on the growth of this species was assessed. To this end, *P. tricornutum* was spiked at a concentration of 10,000 cells mL^{-1} in 100 mL conical flasks containing 50 mL of test medium and uncontaminated PDMS samplers. The samplers

Table 3.3: Physico-chemical characteristics of PAHs. VP: vapour pressure.

	log K_{ow}[a]	log K_d[b]	VP (mmHg)[c]
Naphthalene	3.30	3.03	0.085
Acenaphthene	3.92	3.62	0.00215
Fluorene	4.18	3.79	0.0006
Phenanthrene	4.46	4.11	0.000121
Fluoranthene	5.16	4.62	0.00000922

[a] Measured at 25°C [80]
[b] Measured at 20°C [177]
[c] Measured at 25°C [178–180]

were added in 4 different amounts: 0.5, 1, 1.5 and 2 sheets (the equivalent of 1.57, 3.15, 4.73 and 6.30 g of sampler, respectively). The test flasks – including three control flasks which did not contain samplers – were incubated for 72 hours under the conditions described in Chapter 2. The cell concentrations were measured daily with an electronic particle counter (*Coulter Counter model DN*, Harpenden, Herts, UK). The cell concentration data obtained at 72 hours was used to calculate the percentage of growth inhibition compared to the control flasks. To this end, the specific growth rate μ was calculated as in Chapter 2. The percentage of growth inhibition $I_{\mu i}$ was then calculated as follows:

$$I_{\mu i} = \frac{\bar{\mu}_c - \mu_i}{\bar{\mu}_c} \cdot 100 \tag{3.9}$$

with $\bar{\mu}_c$ as the average growth rate of the control (the flasks without samplers) and μ_i as the growth rate of the individual test flasks.

3.3 Results and Discussion

3.3.1 Modelling

3.3.1.1 Depletion

Results of the modelling exercises indicate that, for 1 g of sampler added to 0.05 L of water, compounds with a log(Kd) lower than 2.7 will be depleted by 10% in the sampler (increasing with decreasing log K_d; Figure 3.2). If 5 g or 10 g of sampler is added, 10% of depletion would occur at a log Kd value of 2.0 or 1.7, respectively. In reality, this depletion is likely a bit higher given that substance losses via evaporation, (bio)degradation, adsorption to test vessel walls and biouptake were not taken into account in this modelling exercise.

A depletion of 10% of a substance in a sampler causes a deviation of 10% from the equilibrium that had been established with the environmental contaminant concentration during the passive sampling period. Hence, the concentration of

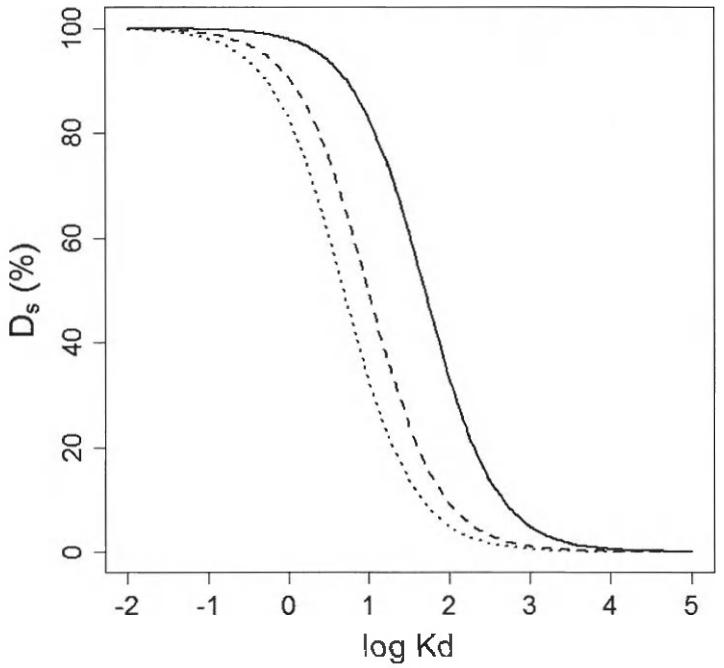

Figure 3.2: Percentage of depletion of chemical compounds in PDMS solid phases (D_s) of different weight after equilibration in 0.05 L of test medium in function of log Kd. Solid line: 1 g of PDMS added, dashed line: 5 g of PDMS added, dotted line: 10g of PDMS added.

substances with a log K_d of 2 (in the case 5 g of sampler is added to 0.05 L of uncontaminated test medium) will be at least 10% lower in the test medium as compared to the average concentrations in the field during the passive sampling period. Moreover, as the log K_d decreases further, this percentage of deviation from equilibrium increases rapidly (see Figure 3.2). As such, the passive dosing setup in this study – which finally aims to use 6.3 g of sampler in 0.05 L of test medium (see Chapter 4) – is most suitable for substances with a log $K_d \geq 2$.

3.3.1.2 Achieving equilibrium

Model calculations resulted in a thickness of the water boundary layer (δ_w) of 1,164 µm. The resulting molar volumes and diffusion coefficients can be found in Table 3.4 as well as the time needed to reach 95% of the equilibrium (T95). The value obtained for δ_w is very high compared to the apparent boundary layer thickness found in other, similar studies (e.g. Ter Laak et al. [172]). However,

δ_w as calculated in this study is largely fictitious [181] and the apparent δ_w is likely to be much lower. As such, the values for T95 obtained in this study can be considered as a worst case estimation. As T95 was less than 16h at most, an equilibration time of 24h was considered to be adequate for the passive dosing experiments to be conducted in Chapter 4.

Table 3.4: Log Kd (from Smedes et al. [177], measured at 20°C), calculated total molar volumes (V_{ix}) and water diffusivities (Dw) of the compounds used in the modeling exercise, and the time needed to reach 95% of the equilibrium (T95).

Substance	log Kd	V_{ix} ($cm^3\ mol^{-1}$)	Dw ($10^{-6}\ cm^2\ s^{-1}$)	T95 (h)
Naphthalene	3.03	108.5	8.37	8.5
Acenaphthene	3.62	125.9	7.67	9.3
Fluorene	3.79	135.7	7.34	9.7
Phenanthrene	4.11	145.4	7.04	10.1
PCB180	7.00	218.1	5.55	12.8
PBDE209	7.00	313.3	4.48	15.9

3.3.2 Equilibrium experiment

Figure 3.3 shows the results of the first experimental passive dosing validation study, indicating equilibrium was reached for both fluorene and fluoranthene after about 2 to 5 hours (i.e. well before 24 hours). However, the target concentration of 2 $\mu g\ L^{-1}$ was not reached for either of the substances. This was most likely due to the fact that the PDMS-water partitioning coefficient Kd used for the calculation of the spiking concentrations, were determined for ultra pure water [177] while the experiments in this study were all conducted in algal test medium with a high salinity (approximately 33 $g\ L^{-1}$). The salting out effect causes the affinity of the sampler for the substance to increase [182], thus lowering the expected aqueous concentration at equilibrium. Fluorene and fluoranthene have a salting out constant[1] (K^S) of 0.267 [184] and 0.364 [185], respectively. Assuming a salinity of 0.56 M NaCl, the adjusted Kd values of these PAHs were roughly a factor of 1.5 higher than the original Kd values. Using these Kd values, the expected aqueous concentrations of fluorene and fluoranthene were 1.27 and 1.12 $\mu g\ L^{-1}$, respectively, i.e. close to the measured concentrations (Figure 3.3).

The results of the second validation experiment indicated that with the exception of naphthalene (the most volatile compound), the concentrations of the PAHs

[1] Salting out is the effect that causes the solubility of a chemical to decrease with increasing salinity. This effect also influences the partitioning behaviour of the chemical. The salting out constant K^S is the slope of the straight line relating the logarithm of the solubility of a substance to the salinity. A Kd value adjusted for salinity can be calculated as $Kd,salt = Kd.10^{K^S.[Salt]}$ with [Salt] as the salt concentration (M) [183].

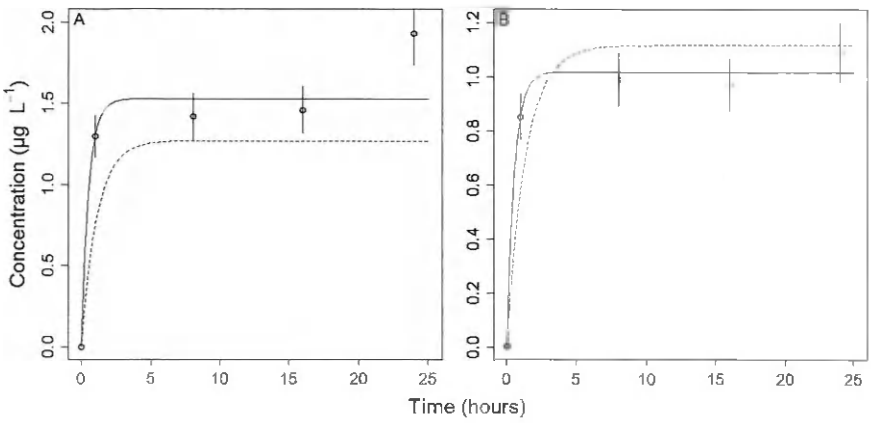

Figure 3.3: Equilibrium kinetics of (A) fluorene and (B) fluoranthene in a 24h passive dosing experiment with polydimethylsiloxane (PDMS) sheets. The test flasks contained 0.05 L of algal test medium and 1.75 g of PDMS sheets loaded with the test substances. All flasks were continuously shaken on a rotary shaker. The error bars represent the analytical error. The dashed curves represent modelled equilibrium kinetics using a one-compartment model with PDMS-water partitioning coefficients (Kd) corrected for the salting out effect and an estimated value for the elimination rate k_e (see Section for more details on the one-compartment model and the derivation of k_e). The solid curves represent a best-fit one-compartment model.

during the exposure period remained within 20% of the concentrations measured at the start of this period. As such, the test setup employed during this experiment was deemed adequate for the purpose of the passive dosing experiments to be conducted in Chapter 4.

3.3.3 Effects of light attenuation by passive samplers on growth of P. tricornutum

Table 3.5 presents the results of the light attenuation test and shows that the average percentage of growth inhibition ($< I_{\mu i} >$) remained below 10 % in all test flasks. Remarkably, the growth inhibition observed in the flask containing the highest number of the PDMS sheets was the lowest. Therefore, the influence of the samplers on the growth of the test species due to light attenuation was considered negligible. Moreover, as in all the test flasks the necessary cell increase of a factor 16 was achieved [77], the samplers were not considered unlikely to influence the results of the ecotoxicity test. In order to account for any potential growth inhibition caused by the PDMS samplers, it was decided to include control flasks containing uncontaminated samplers during all subsequent passive dosing experiments.

Table 3.5: Average percentage of growth inhibition ($< I_{\mu i} >$) observed in a 72h growth inhibition test with P. tricornutum caused by the presence of different amounts of uncontaminated PDMS passive samplers in the test medium.

# of samplers added	$< I_{\mu i} >$ (%)
0.5	4.4
1	7.3
1.5	8.5
2	1.7

3.4 Conclusions

The results of this chapter show that PDMS sheets are able to dose hydrophobic compounds into ecotoxicological test medium and maintain the concentration levels for at least 72 hours. The passive dosing setup used in this thesis is most suitable for compounds with a log $K_d \geq 2$. Less hydrophobic compounds become significantly depleted from the sheets, leading to test concentrations that will be lower than the average contaminant concentrations in the field during the passive sampling period. A potential solution for this problem may be to use a combination of multiple polar and nonpolar sampler types in order to obtain a more realistic exposure of a broader range of chemicals. For monitoring studies, such an approach has already been followed (e.g. Petty et al. [186]).

4

Application of a passive dosing technique to assess the ecotoxicity of realistic environmental contaminant mixtures.

Redrafted from:
Claessens, M., Monteyne, E., Roose, P., Janssen, C.R. Passive sampling reversed: passive dosing as a technique to assess the ecotoxicity of realistic environmental contaminant mixtures. Aquatic Toxicology, submitted.

Abstract

In this chapter, the passive dosing technique developed in Chapter 3 was applied in a 72h algal growth inhibition assay with *P. tricornutum*. The samplers used in this assay had previously been deployed at dedicated locations in the Belgian marine environment. As such, the environmental contaminant mixtures collected by the samplers *in situ* were recreated in the laboratory test medium through passive dosing. The exposure concentrations achieved in the test medium reflected the average concentrations of contaminants present in the field during the period of passive sampling. As such, the mixtures created in the laboratory medium are more representative of general pollution levels than the mixtures – obtained through grab sampling – that are used in whole-sample bioassays. As for the compounds in the higher hydrophobicity range (log $K_{ow} > 4.1$) equilibrium had not yet been reached after the passive sampling period, the concentrations of such compounds in the test medium was lower than their average environmental concentrations. This shortcoming may be solved in the future by using a combination of different passive sampling materials.

For a majority of the mixtures, a low to moderate growth stimulation was observed in the test species. In 4 cases however, severe growth inhibition was observed (i.e. 66–100%). A mixture risk assessment in which contaminant concentrations – obtained through conventional monitoring at the passive sampling stations – of 78 compounds from 8 different chemical groups were used, did not indicate that such severe growth inhibition could be expected in *P. tricornutum*. Potential explanations for the observed growth inhibition include (1) the presence of unknown compounds on the samplers (anthropogenic contaminants and/or marine toxins), (2) the general lack of appropriate ecotoxicity data used for the mixture risk assessment, or (3) experimental artefacts caused by the presence of the samplers.

Overall, the passive dosing methodology presented in this chapter provides a useful tool to expose organisms to realistic environmental contaminant mixtures which can be of practical use in tiered mixture risk assessment approaches.

4.1 Introduction

In Chapter 3, a passive dosing methodology using PDMS sheets was developed. In this chapter, this methodology will be applied using PDMS samplers that have been deployed in at locations in the Belgian marine environment. As such, the mixtures of micropollutants collected *in situ* by passive sampling are recreated in laboratory test medium by reversely using the samplers as dosing devices. The effects of these mixtures on the growth of the marine diatom *P. tricornutum* will be assessed. The observed effects will be interpreted based on measured contaminant concentrations in grab samples.

4.2 Methodology

4.2.1 Passive sampling methodology

Full details on the passive sampling methodology can be found in Monteyne et al. [176]. Briefly, polydimethylsiloxane (PDMS) sheets (AlteSil Laboratory Sheet, Altec Products Ltd, Bude, United Kingdom) of 55 mm x 90 mm and a thickness of 0.5 mm were used as passive sampling devices. The samplers were pre-cleaned by Soxhlet extraction and subsequently mounted in stainless steel cages in a way that ensured they could move freely (as proposed by Smedes [187]). For this study, samplers were deployed 3 times during 2008 and 2009 for approximately 6 weeks at 4 to 8 different locations in the Belgian coastal zone. Of these locations – which partly match the sampling locations described in Chapter 2 – 7 were distributed in 3 coastal harbours and 1 was located approximately 5 miles offshore (Figure 4.1). Table 4.1 shows which of the stations were included in the respective passive sampling campaigns. After the sampling period, the samplers were transported to the laboratory in pre-cleaned, closed glass recipients on ice and stored at -20 ± 1 °C until further use.

Table 4.1: Passive sampling scheme. x: station included, –: station not included.

Period	NP1	NP2	OO1	OO2	OO3	ZB2	ZB3	Sea
Apr–Jul 2008	x	x	x	x	x	x	x	x
Oct–Dec 2008	–	x	–	x	–	x	x	–
Aug–Oct 2009	–	x	–	x	–	x	x	x

4.2.2 Spot-sampling – Methodology and chemical analysis

Full details on the spot sampling methodology can be found in Monteyne et al. [176]. In summary, water samples were collected with 5 or 10L Niskin or Go-Flo bottles depending on the depth at the sampling location. Samples were taken

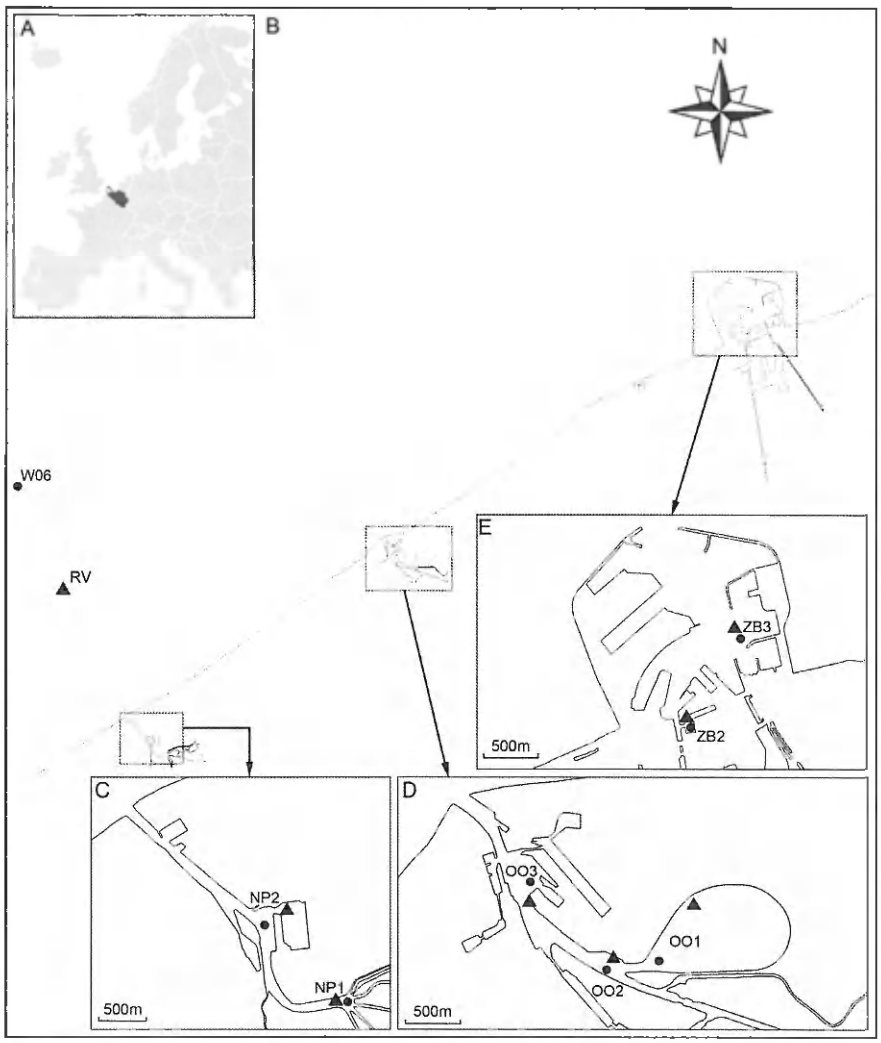

Figure 4.1: The study area with the sampling locations used for the work in this chapter; A. Location of Belgium in Europe; B. Overview of the Belgian coastal zone showing 2 sampling stations (including 1 passive sampling station) on the Belgian Continental Shelf; C. Two sampling stations in the harbour of Nieuwpoort; D. Three sampling stations in the harbour of Oostende; E. Two sampling stations in the harbour of Zeebrugge. ●: station included in the integrated sampling campaigns; ▲: station where passive samplers were deployed.

5 times during 2007–2009 at the passive sampling locations or in close proximity (Figure 4.1). Water samples were analysed for PCBs, PAHs, PBDEs, organotins, organonitrogen pesticides, pharmaceuticals, phenols and phthalates. The analytical methodologies and concentration data are accessible through the INRAM project [188] and have partly been published by Monteyne et al. [176] and Wille et al. [71]. The analytical methods will be described in more detail in Chapter 5.

4.2.3 Passive dosing – Algal growth inhibition

All biofouling was removed from the field-deployed passive samplers by cleaning them with moistened paper. The samplers were subsequently used in a passive dosing study with the marine diatom *Phaeodactylum tricornutum* according to the methodologies described in Chapter 3. To each test flask, 2 samplers were added that were each cut in 4 equal pieces to allow free movement of the sheets during the test. Subsequently, the test flasks were placed on a rotary shaker for equilibration. After 24 hours the test flasks were inoculated with 10,000 cells/mL of the 3 day old *P. tricornutum* culture and incubated under the conditions described in Chapter 2. All flasks were shaken manually three times a day. The algal cell density was measured after 24, 48 and 72 hours using an electronic particle counter (Coulter Counter model DN, Harpenden, Herts, UK). All exposures were conducted in duplicate and control flasks with uncontaminated samplers were included. The specific growth rate μ and the percentage of growth inhibition $I_{\mu i}$ were calculated as described in Chapters 2 and 3.

4.2.4 Mixture risk assessment

To assess the risk of contaminant mixtures towards the test organism *P. tricornutum*, a low tier mixture risk assessment strategy similar to the Tier 0 assessment approaches of Price et al. [48] and the World Health Organization [47] was followed. This stage of risk assessment is typically characterized by data gaps in either the exposure or the (eco)toxicity data. The latter is the case for *P. tricornutum*, for which only limited ecotoxicity data are available. At this tier such data gaps can be filled by using e.g. quantitative structure-activity relationships (QSARs) or read across approaches [48]. In this study, a QSAR was developed for PAHs by linear regression of the octanol-water partitioning coefficient against the ecotoxicity (see Appendix B section B.1). For some of the other chemicals ecotoxicity data for other marine diatoms were used and for some chemicals considered to exert their toxicity via the same mode of action, a (conservative) group EC50 value was used in case data points were missing (Table 4.2). As analytical data of the passive samplers are at this point limited to PAHs and PCBs [176] and to few pesticides and pharmaceuticals [131], it was not possible to determine the true exposure concentrations achieved in the passive dosing tests with *P. tri-*

cornutum for an extended set of chemicals. Therefore, it was decided to use the exposure data from the analysis of the water samples collected during the 5 sampling campaigns during 2007–2009. For each sampling location, the minimum and maximum concentrations of each of the chemicals observed over the 3 years were used in a simple Monte-Carlo analysis to generate 100,000 sets of hypothetical concentrations per station for pharmaceuticals, organotins, pesticides, PAHs, PCBs, PBDEs, phenols, phthalates and PFCs. In this low tier mixture risk assessment, uniform distributions were used for this analysis as a conservative approach. For each of these 100,000 sets, the hazard index (HI) was calculated as follows:

$$HI = \sum \frac{C_{wi}}{EC50_i} \tag{4.1}$$

in which C_{wi} and $EC50_i$ are the water concentration (as measured by spot sampling) and EC50 (the concentration causing 50% effect, in this case 50% algal growth inhibition) of the i^{th} compound in the mixture, respectively. If a mixture exhibits an HI value of 1, it is expected to cause 50% effect. This Monte-Carlo analysis resulted in ranges of possible HI values for each location which were then compared to the test results obtained in the passive dosing experiments with *P. tricornutum*. The HI ranges were also calculated for the different compound groups separately to be able to estimate their respective contributions to the total mixture toxicity.

Table 4.2: Log K_{ow} values and ecotoxicity to marine diatoms of the compounds included in this study. PT: Phaeodactylum tricornutum, CS: Chaetoceros socialis, SC: Skeletonema costatum, TG: Thalassiosira guillardii, NC: Nitzschia closterium, NI: Navicula incerta, DMP: dimethyl phthalate.

Compound	log K_{ow}	Test duration & species	EC50	Reference
Pharmaceuticals				
Salicylic acid	2.26	72h PT	255.5 mg L^{-1}	Chapter 2
Paracetamol	0.46	72h PT	265.8 mg L^{-1}	Chapter 2
Carbamazepine	2.45	72h PT	62.5 mg L^{-1}	Chapter 2
Atenolol	0.16	72h PT	877 mg L^{-1}	Chapter 2
Propranolol	3.48	72h PT	0.288 mg L^{-1}	Chapter 2
Trimethoprim	0.91	72h PT	5.1 mg L^{-1}	Chapter 2
PCBs	5.6–7.92	72h CS	7.0 µg L^{-1}	[189]
Pesticides				
alachlor	3.52	96h SC	3.4 µg L^{-1}	Acetochlor value [190]
atrazine	2.61	96h PT	60.6 µg L^{-1}	[191]
azoxystrobin	2.5	120h SC	453 µg L^{-1}	[190]
carbendazim	1.52	96h TG	19056 µg L^{-1}	[190]
chloridazon	1.14	120h SC	1030 µg L^{-1}	[190]
chlortoluron	2.41	72h PT	21 µg L^{-1}	Diuron value
cyanazine	2.22	120h SC	17.8 µg L^{-1}	[190]
diuron	2.68	72h PT	21 µg L^{-1}	[191]
flufenacet	3.2	120h SC	5 µg L^{-1}	[190]
isoproturon	2.87	72h PT	53.1 µg L^{-1}	[191]
linuron	3.2	120h SC	35.9 µg L^{-1}	[190]
metazachlor	2.13	96h SC	3.4 µg L^{-1}	Acetochlor value [190]

Continued on next page

Table 4.2 – continued from previous page

Compound	log K_{cw}	Test duration & species	EC50	Reference
methabenzthiazuron	2.64	72h PT	21 µg L^{-1}	Diuron value
metolachlor	3.13	120h SC	61 µg L^{-1}	[190]
monolinuron	2.3	72h PT	21 µg L^{-1}	Diuron value
propachlor	2.18	96h SC	3.4 µg L^{-1}	Acetochlor value [190]
simazine	2.18	96h PT	101.3 µg L^{-1}	Calculated
terbutylazine	3.21	96h PT	34.4 µg L^{-1}	Calculated
acetochlor	3.03	96h SC	3.4 µg L^{-1}	[190]
PAHs				
naphthalene	3.3	72h PT	41986 µg L^{-1}	Calculated
1-methylnaphthalene	3.87	72h PT	8265 µg L^{-1}	Calculated
acenaphthene	3.92	72h PT	7167 µg L^{-1}	Calculated
acenaphthylene	3.94	72h PT	6770 µg L^{-1}	Calculated
biphenyl	4.01	72h PT	5545 µg L^{-1}	Calculated
Fluorene	4.18	72h PT	3991 µg L^{-1}	This study
2,6-dimethylnaphthalene	4.31	72h PT	2357 µg L^{-1}	Calculated
2,3,5-trimethylnaphthalene	4.36	72h PT	2046 µg L^{-1}	Calculated
Anthracene	4.45	72h PT	1753 µg L^{-1}	This study
Phenanthrene	4.46	72h PT	1183 µg L^{-1}	This study
Pyrene	5.22	72h PT	358 µg L^{-1}	This study
1-methylphenanthrene	5.08	72h PT	262 µg L^{-1}	Calculated
Fluoranthene	5.16	72h PT	103 µg L^{-1}	[192]
benzo(a)anthracene	5.79	72h PT	34 µg L^{-1}	Calculated
chrysene	5.81	72h PT	32 µg L^{-1}	Calculated
benzo(a)pyrene	5.97	72h PT	20 µg L^{-1}	Calculated
benzo(e)pyrene	6.11	72h PT	14 µg L^{-1}	Calculated
benzo(b)fluoranthene	6.11	72h PT	14 µg L^{-1}	Calculated
benzo(k)fluoranthene	6.11	72h PT	14 µg L^{-1}	Calculated
dibenz(a,h)anthtracene	6.5	72h PT	4 µg L^{-1}	Calculated
indeno(1,2,3-cd)pyrene	6.62	72h PT	3 µg L^{-1}	Calculated
benzo(ghi)perylene	6.63	72h PT	3 µg L^{-1}	Calculated
Organotins				
TBTO	3.84	72h PT	0.83 µg L^{-1}	[191]
DBTCl2	1.56	72h SC	40 µg L^{-1}	[193]
TPTCl	4.19	72h PT	0.93 µg L^{-1}	[191]
DPTCl2	1.38	72h SC	31 µg L^{-1}	[193]
PBDEs				
PBDE 47	6.77	48h SC	70 µg L^{-1}	[194]
Phenols				
2,4,6-trichlorophenol	3.69	72h NC	4900 µg L^{-1}	[195]
2,4-dichlorophenol	3.06	72h PT	600 µg L^{-1}	[196]
4-chlorophenol	2.39	96h PT	9600 µg L^{-1}	[197]
Bisphenol A	3.32	96h SC	1000 µg L^{-1}	[198]
Phenol	1.46	72h PT	9586 µg L^{-1}	This study
Pentachlorophenol	5.12	96h PT	3000 µg L^{-1}	[199]
nonylphenol	5.76	96h NI	200 µg L^{-1}	[200]
4-chloro-3-methylphenol	3.1	72h PT	7910 µg L^{-1}	This study
Phthalates				
Diehtylphthalate	2.42	96h PT	120 µg L^{-1}	Value for DMP [201]
Dipentylphthalate	5.62	96h PT	120 µg L^{-1}	Value for DMP [201]
Butylbenzylphthalate	4.73	96h PT	120 µg L^{-1}	Value for DMP [201]
bis-(2-ethylhexyl)phthalate	7.60	96h PT	120 µg L^{-1}	Value for DMP [201]

4.3 Results and Discussion

The results of the passive dosing experiments in which the marine diatom *P. tricornutum* was exposed to environmental contaminant mixtures are presented in

Table 4.3. In most cases (12 out of 17) a low to moderate growth stimulation was observed, indicating no adverse effects at these sampling stations. A possible explanation for the observed growth stimulation is the moderate stimulatory effect compared to the control organisms (30–60% at maximum according to Calabrese [202]) that is witnessed in some (eco)toxicity studies at the lower dose(s). This phenomenon – which is generally termed 'hormesis' – is frequently observed in growth inhibition tests with algae. Stebbing [203] and Davoren et al. [204] found a stimulatory response from the marine diatom *Skeletonema costatum* following exposure to a sediment elutriate.

For the first passive sampling period (April-July 2008), a significant growth inhibition of 100, 66 and 100% were observed for stations OO1, OO2 and ZB2, respectively (Table 4.3). Station ZB3 exhibited 100% growth inhibition in 2009. Thus, although growth stimulation appeared to be the most common effect observed in this study, some field mixtures caused severe adverse effects on the test organism.

Table 4.3: Average percentage of growth inhibition of P. tricornutum observed after 72h of exposure to environmental contaminant mixtures administered through passive dosing. A negative percentage indicates growth stimulation compared to the control exposure. Values in brackets indicate the standard deviation.

	NP1	NP2	OO1	OO2	OO3	ZB2	ZB3	RV
Apr–Jul 2008	-8(2)	-25(4)	100(0)	66(26)	-6(5)	100(0)	-27(1)	0(–)
Oct–Dec 2008	–	-19(4)	–	-17(2)	–	-24(2)	-23(3)	–
Aug–Oct 2009	–	-37(3)	–	-36(1)	–	-49(1)	100(0)	-43(1)

In order to investigate to what extent the contaminant mixtures at the passive sampling stations could explain the observed results, aquatic concentration data from spot sampling campaigns were used to generate the possible range of the hazard index (HI) of these mixtures via a simple Monte-carlo analysis. This was based on the ranges of all 94 compounds as observed at the separate sampling stations over the three years of spot sampling campaigns. The results of this exercise are represented as boxplots of the HI in Figure 4.2. These data clearly show that the maximum values of HI are well below 1 – the value at which 50% growth inhibition of *P. tricornutum* would be expected – at all sampling stations. While these results seem to be in line with the majority of the passive dosing experiments that exhibited growth stimulation rather than inhibition, they clearly fail to explain the more severe adverse effects that were observed. This observation is especially remarkable as the concentration data obtained by spot sampling and analysis are generally higher than the freely dissolved contaminant concentrations reflected by passive sampling. As such we expected the HI obtained via the spot sampling data to be an overestimation. This apparent discrepancy could be due to a number of factors. First of all, there could be unidentified compounds present on the

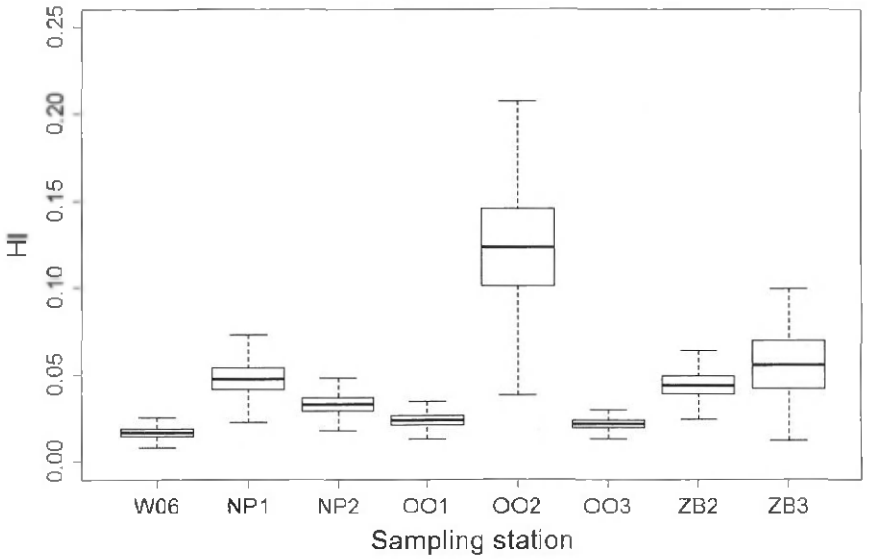

Figure 4.2: Boxplots of the hazard index (HI) obtained via Monte-Carlo analysis of the mixtures from the individual sampling stations.

passive samplers. Such compounds could be either anthropogenic or natural, with algal toxins as examples of the latter. Indeed, some of those toxins are lipophilic and have been monitored by passive sampling (e.g. Mackenzie et al. and Fux et al. [205, 206]). Although at this point it is not known whether they can also accumulate in PDMS samplers, some algal toxins have been found to exert a toxic effect on plankton communities and algal monocultures [207, 208]. Considering unknown mixture components, it is not unusual for a large fraction of complex environmental mixtures to be chemically uncharacterized, leaving a potentially toxic portion unidentified [209]. A potential solution for this problem could be the use of time-of-flight (ToF) and Orbitrap instruments that allow the accurate mass screening of a virtually unlimited number of analytes, both targeted and untargeted [30, 210, 211].

Another possibility for the occurrence of severe effects is that *P. tricornutum* is more sensitive to some compounds than the marine diatoms of which the ecotoxicity data were used for the mixture risk assessment. For example, for 11 of the 19 pesticides included in this study, the ecotoxicity data originated from experiments with a diatom other than *P. tricornutum*. This could cause an underestimation of the adverse effects if *P. tricornutum* is more sensitive to these compounds. Moreover, the group ecotoxicity values used for some compounds (e.g. PCBs and PBDEs) could have caused an underestimation of the true toxic effects in a similar

way. Generating additional ecotoxicity values for *P. tricornutum* will be important to take the mixture risk assessment to a higher level.

As there are virtually no studies available that investigate the effects of mixtures on *P. tricornutum*, it is also not possible to rule out the occurrence of synergistic effects. Synergistic mixture effects occur when two constituents interact to produce a more than additive effect. However, synergy is in general recognized to be a rare phenomenon that mainly occurs if the interacting constituents are present at high concentrations [40] and its occurrence has been found to decrease when the number of mixture components increases [212]. As such it is rather unlikely that this phenomenon is responsible for the severe effects observed for some of the mixtures tested here.

Lastly, as the passive samplers partially shield the algae from their light source, experimental artefacts may be responsible for the observed effects. However, if this would be a consistent effect then severe growth inhibition should have been observed in all test flasks (including the controls which all contained an uncontaminated samplers) rather than be limited to a few. Moreover, as a previous experiment has pointed out that the presence of samplers in the test medium has only a very minor effect on the algal growth (see Chapter 3), such artefacts can be excluded.

Table 4.4 presents the average contributions of the different contaminant groups to the HI for the individual sampling stations. The pesticides are the most dominant group at all stations except ZB2 where PAHs contribute the most to the total mixture toxicity. As the test species is a diatom and the group of pesticides contain a number of herbicides, this was not unexpected. In general, pesticides, PAHs and organotins were responsible for at least 90 % of the mixture toxicity observed at all sampling stations. However, since – as noted above – the more severe observed effects in the passive dosing studies could not be explained by the HI values, these observations will likely change when the mixture risk assessment is refined.

Table 4.4: Average theoretical contribution of the different pollutant groups to the mixture toxicity, expressed as percentage of the total mixture hazard index (HI). Bold values indicate the most dominant fraction.

	Pharmaceuticals	Organotins	Pesticides	PAHs	PCBs	PBDEs	Phenols	Phthalates	PFCs
W06	0.0	31.1	**50.7**	13.9	0.6	0.1	2.5	0.9	0.3
NP1	0.1	14.7	**72.0**	8.4	0.2	0.1	1.0	3.0	0.1
NP2	0.1	26.8	**52.7**	15.6	0.9	0.1	2.5	1.2	0.2
OO1	0.1	17.3	**65.9**	12.4	0.8	0.3	1.7	1.2	0.4
OO2	0.1	8.7	**85.5**	3.9	0.6	0.0	0.4	0.7	0.2
OO3	0.1	31.0	**51.7**	11.1	1.1	0.1	1.9	2.6	0.4
ZB2	0.0	24.3	29.0	**36.5**	0.8	0.5	0.9	7.8	0.2
ZB3	0.0	16.4	**59.4**	17.4	0.2	0.3	0.8	5.4	0.1

The method presented here (*i.e. passive dosing of field mixtures collected by* passive sampling) has an important advantage over the experiments in which test organisms are exposed to passive sampler extracts. Nonpolar passive samplers (SPMDs in particular) are in general regarded to mimick uptake of hydrophobic contaminants in organisms fairly well (e.g. Huckins et al. [213]). Indeed, the concentration of a contaminant in a passive sampler or its extract (as obtained by solvent extraction), is more representative of the body burden of that substance rather than of its external exposure concentration. For this reason the concentration ratio of two compounds with a different hydrophobicity will certainly be different in the passive sampler extract compared to their concentration ratio in the aqueous environment from which they were sampled. As such, exposing organisms to field mixtures by spiking passive sampler extracts directly into the test medium, is not environmentally realistic. The only way to reestablish the original concentration ratios of contaminants in the test medium, is to use the passive dosing approach proposed in this study.

Our approach also has some shortcomings in its current form. The first one was described in Chapter 3 and pointed out that depletion of substances with a log $K_d < 2$ leads to lower concentrations of these chemicals in the test medium compared to their environmental levels at the time of passive sampling. Secondly, the compounds in the higher hydrophobicity range did not always reach equilibrium after the general sampling period of 6 weeks. This could be deduced from the dissipation of performance reference compounds (PRCs) that were spiked on separate sets of passive samplers deployed simultaneously with the samplers used in this study. The samplers loaded with PRCs were used in the study of Monteyne et al. [176] in which freely dissolved concentrations of PAHs and PCBs were derived based on PRC dissipation. The results of that study pointed out that on average, PRCs with a log K_d of 4.1 had dissipated from the samplers for 90%. For PRCs with a log K_d of 4.6 and 5.4, this was only 50% and 10%, respectively (see Appendix B section B.2 for more details). As such, for substances with a log K_d of 4.6, equilibrium was only established for 50%, meaning that their concentration in the test medium achieved through passive dosing would be only half of the average environmental concentration present during the sampling period. Similarly, for substances with a log K_d of 5.4, the concentration in the test medium would only be 10% of the average concentration in the environment. The use of a combination of multiple polar and nonpolar sampler types which was suggested in Chapter 3 to resolve the former shortcoming, may also be helpful to overcome the latter.

4.4 Conclusions

In this chapter, a new methodology was applied in which the test organism *P. tricornutum* could be exposed – via passive dosing – to contaminant mixtures that were previously collected in the field through passive sampling. The majority of the mixtures did not cause adverse effects on the test species. Some mixtures, however, caused severe growth inhibition which could not be explained based on exposure information obtained through conventional monitoring. This was most likely due to the presence of unknown chemicals on the passive samplers. The exposure concentrations were environmentally relevant and expected to be constant during the test period due to the passive dosing approach. In the lower ($\log K_d < 2$) and higher ($\log K_d > 4$) hydrophobicity range, a good reflection of the environmental contaminant levels could not yet be guaranteed. In future studies, the inclusion of other types of passive samplers can be considered to overcome this finding.

5

Modelling the fate of micropollutants in the marine environment using passive sampling: an equilibrium partitioning approach.

Redrafted from: Claessens, M., De Laender, F., Monteyne, E., Roose, P., Janssen, C.R. Modelling the fate of micropollutants in the marine environment using passive sampling. Chemosphere, to be submitted.

Abstract

Polydimethylsiloxane sheets were used to determine freely dissolved concentrations (C_{diss}) of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the Belgian coastal zone. C_{diss} and relevant partitioning coefficients from literature were subsequently used as the key input parameters in equilibrium models to predict the whole water concentrations (C_{ww}) of these compounds as well as their concentrations in sediment, suspended particulate matter (SPM) and biota. The modelling results were compared to analytical data obtained through conventional chemical analysis of grab samples from the same area.

In general, contaminant concentrations were predicted well for whole water and biota. C_{ww} was increasingly underpredicted as K_{oc} increased, possibly because of the presence of black carbon. Concentrations in biota were overestimated by the equilibrium approach when log K_{ow} exceeded 6.5, suggesting an increasing role of transformation processes. Concentrations of PAHs and PCBs in sediment and SPM were consistently underpredicted although a good correlation between measured and predicted values was observed. This was potentially due to the use of experimental K_{oc} values which have been found to underestimate partitioning of hydrophobic substances to sediment in field studies.

Overall, the results showed that a passive sampling approach combined with a simple equilibrium partitioning model can function as a baseline model to predict contaminant concentrations in environmental matrices.

5.1 Introduction

For the environmental and human health risk assessment of chemicals in the aquatic environment, reliable concentration data of chemicals in water, sediment and biota are indispensable. However, the monitoring and analysis of chemicals in these compartments continues to represent a significant challenge. Indeed, using conventional grab samples, a relatively large number of samples is needed for a given sampling area to obtain reliable and meaningful exposure data [135, 214]. Such a sampling approach is time consuming and can be very costly [215] and the chemical analysis often requires difficult extraction and clean-up techniques [12].

One option to reduce this monitoring effort is to obtain data on freely dissolved concentrations of contaminants. Such data can then be used to predict the partitioning of these compounds to other compartments (e.g. sediment and biota) [151, 216, 217]. However, many nonpolar organic substances such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) readily sorb to sediments and suspended particulate matter (SPM), causing their dissolved concentrations to be in the low ng L^{-1} to pg L^{-1} range which makes them difficult to quantify [218]. Moreover, surface water generally also contains dissolved organic carbon (DOC) which is – unlike SPM – not separated from the water sample by the conventional filtration techniques. As hydrophobic compounds (e.g. PAHs and PCBs) bind to DOC, the fraction regarded as "dissolved" concentrations of such compounds in reality still consists of a freely dissolved and a DOC-bound fraction [52].

To measure freely dissolved concentrations of contaminants more directly without interference by the DOC-bound fraction, passive sampling devices can be used [53]. Examples of such samplers include the bi-phasic semipermeable membrane devices (SPMDs) which have been used since the 1990's [57]. Many single-phase materials such as polydimethylsiloxane (PDMS), low-density polyethylene (LDPE) and polyoxymethylene show a high affinity for hydrophobic compounds as well, are cheaper and easier to use than SPMDs and they have the possibility to be reused [53, 151, 219]. Moreover, passive samplers integrate the contaminants over the exposure time which makes it a technique that is much less sensitive to accidental, extreme variations of contaminant concentrations [214]. In a number of studies, it has already been attempted to compare and correlate contaminant concentrations in passive samplers (mostly SPMDs) to those in biota [220–228] and to a lesser extent in sediment [223, 228]. In these studies, uncontaminated biota (often bivalves) were caged and deployed in parallel with the passive samplers.

The goal of this chapter is to evaluate if dissolved aqueous contaminant concentrations of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) derived from passive sampling can be used to obtain reliable estimates of contaminant concentrations in different marine compartments: i.e. the whole water phase, sediment, SPM and biota. The predicted concentrations are compared with analytical data obtained through the conventional chemical analysis of grab samples from the same area where the passive samplers were deployed. The suitability of equilibrium models is discussed for each compartment.

5.2 Methodology

5.2.1 Conventional sampling and chemical analysis

Detailed information on the sampling methods and the subsequent chemical analysis can be found in Monteyne et al. [176] and Claessens et al. [188]. Briefly, water, sediment, suspended particulate matter (SPM), shrimp (*Crangon crangon*) and flatfish (*Limanda limanda*, muscle tissue and liver separately) were sampled at 6 offshore locations on the Belgian Continental Shelf. Additional water and sediment samples were taken at different locations in three Belgian coastal harbours. All sampling stations are represented in Figure 5.1. Full sampling campaigns were conducted in 2007, 2008 and 2009.

Water samples were extracted using solid-phase extraction. Sediment samples were centrifuged with a flow-through centrifuge (Biofuge Stratos Heraeus, Kendro Laboratory Products, Hanau, Germany) to obtain the clay fraction (<63 μm), biota samples were homogenized with a dispersion tool (IKA Ultra-Turrax®T25 Basic, Staufen, Germany). All solid material samples were then freeze dried with a Christ LMC-2 (Osterode, Germany), milled and homogenized with a Fritsch Pulverisette (Idar-Oberstein, Germany) and subsequently extracted using pressurised liquid extraction. Extracts were cleaned up by adsorption chromatography on alumina with AlOx and compounds were eluted with hexane. All extracts were analysed for PAHs with GC/MS (Thermoquest, Rodano, Milan, Italy) and for PCBs by GC/MS/MS (Thermofinnigan, Austin, Texas, USA). A full list of the analysed substances including physicochemical characteristics is available in Appendix C.3.

The dissolved organic carbon (DOC) content of the water samples was determined as described by Heininger et al. [229]. The sample was automatically injected and pumped through a Skalar continuous flow chain (Skalar Analytical, Breda, The Netherlands). A known ratio of potassium hydroxide and disodiumtetraborate were added after which the sample was led through a Quartz tube coiled around a UV-lamp followed by the addition of sulphuric acid and heating to 97°C. The acid was subsequently neutralized by the addition of sodium hydroxide in

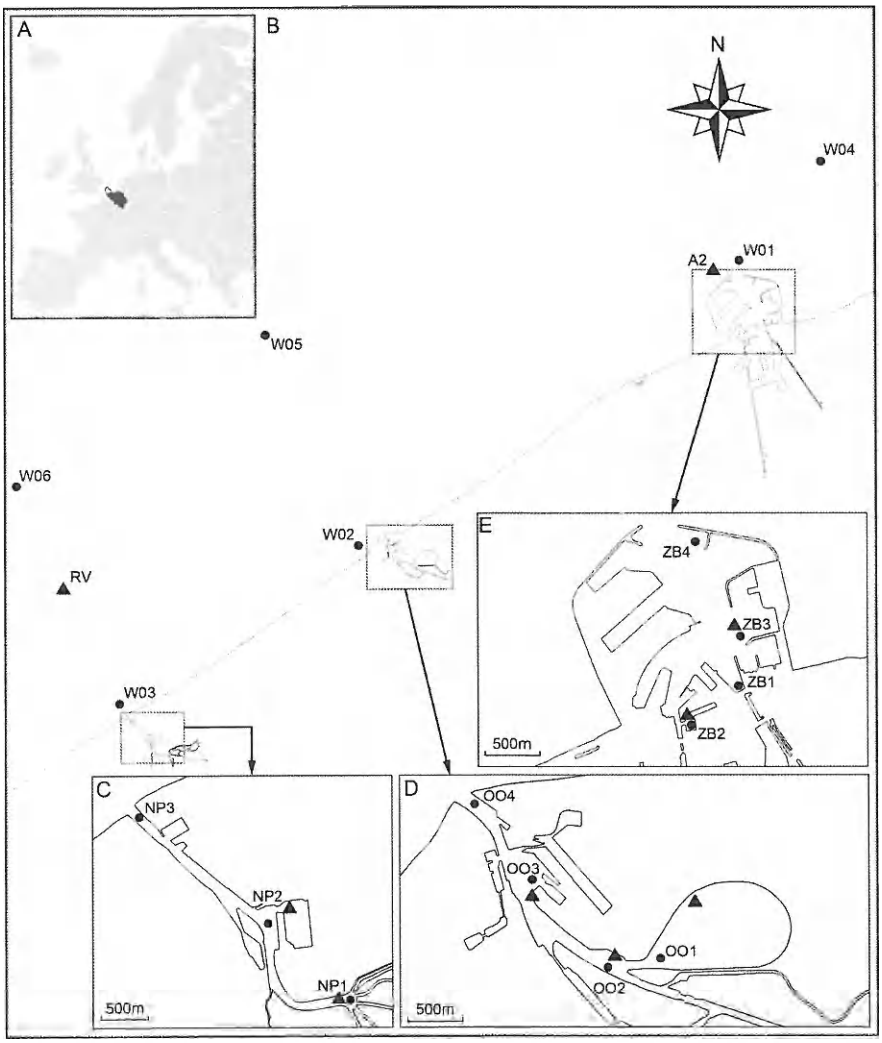

Figure 5.1: The study area with the sampling locations used for the work in this chapter; A. Location of Belgium in Europe; B. Overview of the Belgian coastal zone showing eight sampling stations (including 2 passive sampling stations) on the Belgian Continental Shelf; C. Three sampling stations in the harbour of Nieuwpoort; D. Four sampling stations in the harbour of Oostende; E. Four sampling stations in the harbour of Zeebrugge. ●: station included in the integrated sampling campaigns; ▲: station where passive samplers were deployed.

the presence of ascorbic acid to neutralize chlorine. A known ratio of molybdate and ascorbic acid were added to a sample of the resulting solution and heated to 40°C, causing colour formation. Finally, the extinction of the sample was measured at 880 and 1010 nm with a matrix photometer. Organic carbon contents of sediment and SPM were determined with a flash element analyzer (Thermoquest, Milano, Italy), using the principle of catalytic oxidation followed by gas chromatography [229]. The carbon in the samples is transformed into carbon dioxide in the presence of pure oxygen and tungstenoxide. Subsequently, water is removed from the CO_2-gas by passing it through a magnesium perchlorate column. The CO_2-gas is then separated from nitrogen gas on a packed GC column and detected with a thermal conductivity detector. Glycine was used as a standard.

To determine the lipid contents of the organisms, the lipid weight was measured by pressurized liquid extraction followed by a drying step. Freeze dried biota was extracted using an Accelerated Solvent Extractor (ASE) (Dionex, California, USA). The ASE was used with 100 % of dichloromethane with purity for organic residue analysis as solvent. Extraction cells of 11 mL containing 1 g of biota were filled with solvent and heated within 5 min to 100°C. The materials were extracted with 2 static cycles of 5 min. Between each static cycle 60 % of the solvent was renewed. At the end of the extraction, the cells were rinsed with solvent and purged with nitrogen. The extract was collected in a pre-weighed vial and then dried in an oven at 50°C until constant weight was attained. Based on the final weight of the vial, the lipid content was calculated and expressed as a percentage of the dry weight.

5.2.2 Passive sampling

Full details on the passive sampling methodology can be found in Monteyne et al. [176]. PDMS sheets (the same type as described in Chapter 4) were precleaned by soxhlet extraction with ethylacetate and subsequently spiked with performance reference compounds (PRCs) according to the method described by Booij et al. [55]. From 2007 to 2009, passive samplers were deployed annually as part of the integrated sampling campaigns. In addition, passive samplers were deployed in parallel with caged mussels as part of 2 biomarker experiments conducted in 2008 and 2009 [188]. A full sampling scheme can be found in Appendix C, section C.1. The tissue of the mussels used in these experiments was analysed for PAHs and PCBs according to the procedures described above. For each passive sampling campaign, samplers were deployed at 4 to 7 stations for six to eleven weeks in a stainless steel cage. All stations used for passive sampling are represented in Figure 5.1. After retrieval, the samplers were cleaned to remove biofouling and subsequently extracted by soxhlet extraction using a 1:3 acetone-hexane (*v/v*) so-

lution. Extracts were analysed for PAHs and PCBs by GC/MS.

Freely dissolved water concentrations of PAHs and PCBs (C_{diss} expressed in μg L^{-1}) were calculated using the sampling rate R_s (L d^{-1}) according to the nonlinear least squares method described by Booij and Smedes [56]. The latter was derived from dissipation rates of the PRCs following the methodology of Rusina et al. [230]. More details on the derivation of C_{diss} can be found in Monteyne et al. [176]. An overview of the analytical data availability, see Appendix C section C.2.

5.2.3 Modelling

The freely dissolved water concentration data of PAHs and PCBs obtained with passive sampling was used as input in a simple equilibrium model to predict the concentrations of these compounds in sediment, SPM and biota as well as their whole water concentrations (C_{ww}). In order to calculate C_{ww}, the following formula was used:

$$C_{ww} = C_{diss}(1 + K_{oc} \cdot [DOC] + K_{oc} \cdot [POC]) \tag{5.1}$$

where C_{diss} is the freely dissolved concentration in seawater as derived from passive sampling (μg L^{-1}), K_{oc} is the organic carbon-water partitioning coefficient in L kg^{-1}, and [DOC] and [POC] are the concentrations of DOC and POC (particulate organic carbon) in seawater (kg L^{-1}), respectively.

Concentrations in sediment and SPM were calculated as:

$$C_{sol} = C_{diss} \cdot K_{ow} \cdot f_{oc,sol} \tag{5.2}$$

where C_{sol} is the concentration of pollutants in solids (either sediment or SPM, μg kg^{-1}) and $f_{oc,sol}$ is the fraction of organic carbon in the solids.

Concentrations in biota were calculated as:

$$C_{biota} = C_{ww} \cdot K_{ow} \tag{5.3}$$

where C_{biota} is the concentration in biota (either shrimps, mussels, flatfish liver or flatfish muscle tissue, μg kg lipid^{-1}) and K_{ow} is the octanol-water partitioning coefficient (L kg^{-1}). A list of the used values for K_{oc} and K_{ow} is available in Appendix C section C.3.

To investigate the performance of the models for each compartment, the model bias (MB) was calculated as:

$$MB = 10^{\frac{\sum_{i}^{n} log\frac{Predicted(i)}{Observed(i)}}{n}} \quad (5.4)$$

where Predicted(i) is the concentration predicted by the model in the respective compartment at a given location and time, Observed(i) is the corresponding measured concentration and n is the number of observations. Additionally, the accuracy of the model predictions was assessed by calculating the percentage of predicted data falling within a certain factor of the observed data. When a compound was not detected by conventional monitoring in a certain compartment, its concentration was set at half the detection limit. It is important to note that while in the harbours the passive sampling stations are in close proximity of the stations used for the conventional sampling campaigns, this is not the case for the offshore stations. Indeed, as passive sampling station RV is situated roughly halfway in between stations W03 and W06 (Figure 5.1), there is no conventional sampling station available for direct comparison. For this reason, median measured values from the offshore stations were used for comparison with the predicted data from stations A2 and RV. As the open sea can be considered as a more homogeneous mass than the water bodies within the strongly enclosed harbours, this approach was not expected to generate any additional mismatches.

All modelling was performed using the software R [231].

5.3 Results and Discussion

5.3.1 Whole water concentrations

The equilibrium model predicted 66% and 79% of the measured data within a factor of 5 and within an order of magnitude, respectively (Figure 5.2 A). When interpreting these data, it is important to realise that the water concentrations measured using conventional methods only represent a single point estimate and therefore show a much higher degree of variability than the time-weighted average (TWA) concentrations obtained by passive sampling. This variability is, in a short time frame, due to tidal action and causes differences in contaminant concentrations of up to a factor of 16 [232, 233]. On a longer term, variability may also be due to other factors like weather conditions (e.g. heavy rainfall) and temporal differences in emissions of the contaminants. Further analysis of model-data deviations (Figure 5.2 B) revealed that the whole water concentrations were increasingly underpredicted as the K_{oc} value of the compounds increased. Different factors may explain this trend. Firstly, to model partitioning of compounds to DOC and POC specific partitioning coefficients (i.e. K_{DOC} and K_{POC}, respectively) are often used instead of K_{oc} from which they differ (e.g. [234, 235]). Thus, an attempt to

eliminate the model bias was made by using K_{DOC} and K_{POC} values to model partitioning of PAHs and PCBs to DOC and POC, respectively (see Appendix C.4). In a regression analysis log K_{DOC} and log K_{POC} showed significant linear relations with log K_{oc}, with slopes of 1.19 and 1.11, respectively (see Appendix C.4). As such, using K_{DOC} and K_{POC} as refined estimates of K_{oc} should improve the model's accuracy with increasing log K_{oc}. Indeed, the use of these parameters made this bias less pronounced (see Appendix C section C.5) but the resulting model bias of 0.29 indicated a stronger overall underprediction. Monteyne et al. [176] – who performed a similar, less extensive modelling exercise on the data used in this study – suggested that the presence of black carbon (BC) in the water column may be responsible for the underprediction of whole water concentrations. As data on BC concentrations were not available in this study, the fraction of compounds sorbed to BC could not be taken into account. Similar as for log K_{DOC} and log K_{POC}, the BC-water partitioning coefficient log K_{BC} exhibits a slope of 1.10 in relation with log K_{oc} (see Appendix C.4) and inclusion of BC in the equilibrium model may as such contribute to reduce the observed trend in the model bias. While the fraction of BC in SPM is in general more than a factor of 10 lower than the fraction of OC [236], K_{BC} is on average a factor of 37.4 ± 27.4 higher than K_{oc} for the substances in this study. As such, BC should be included in any future modelling efforts of this kind.

5.3.2 Concentrations in sediment and SPM

The equilibrium model performed poorly for sediment and SPM. The model bias (MB) indicated that the model generally underestimated both the concentrations in sediment and SPM by a factor of 50. Only about 10% of the predicted values were accurate within an order of magnitude for both matrices (Table 5.1). Figures 5.3A and B confirm this bias graphically but nevertheless also indicate a relatively strong relationship between the observed and predicted data. Indeed, statistical analysis of the data yielded Pearson correlation coefficients (PCC) of 0.66 ($N = 322$, $p < 0.001$) and 0.73 ($N = 48$, $p < 0.001$) between the measured and predicted data for sediment and SPM, respectively. The slopes of the regression lines were 0.86 ± 0.06 and 0.70 ± 0.10 for sediment and SPM, respectively (Figure 5.3). A possible cause of the observed consistent underestimation is the tendency of literature values of K_{oc} to be an underestimate of field K_{oc} values. This was for example observed by Hawthorne et al. [237], who found that field K_{oc} values were typically up to two orders of magnitude higher than literature values. In their review on the sorption of organic compounds to different carbon types in sediments and soils, Cornelissen et al. [238] confirm this phenomenon for many different aquatic environments and multiple compound classes (including PAHs and PCBs). This was also observed in laboratory measurements of K_{oc} of PAHs and PCBs in which

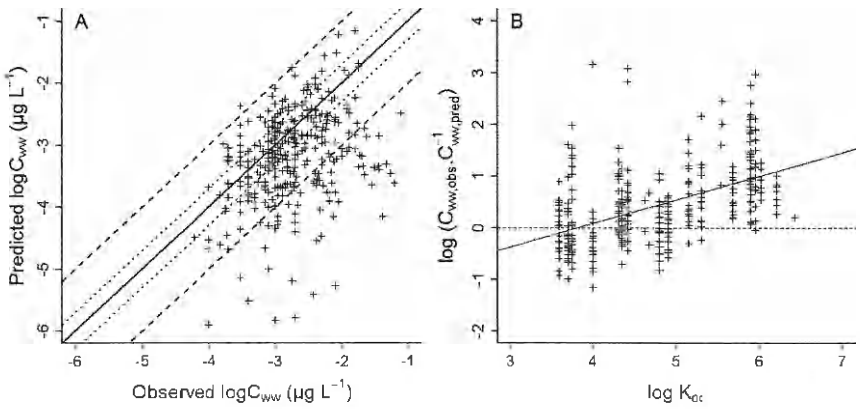

Figure 5.2: Equilibrium model results for the prediction of whole water concentrations of PAHs and PCBs based on literature K_{oc} values and freely dissolved concentrations of these compounds as derived from passive sampling. A: Measured versus predicted whole water concentrations of PAHs and PCBs. The solid line represents the 1:1 relationship, dotted and dashed lines represent a deviation of a factor of 2 and 10, respectively. B: The logarithm of the ratio of observed and predicted concentration data in water vs. log K_{oc}. The solid line represents the regression line (log ($c_{ww,obs}.c^{-1}_{ww,pred}$) = 0.46 log K_{oc} - 1.74, N = 324, R^2 = 0.243, $p < 0.001$). Where log ($c_{ww,obs}.c^{-1}_{ww,pred}$) equals zero (dashed line), observed and predicted data are equal.

solid phase microextraction (SPME) was used to monitor freely dissolved concentrations of the organic chemicals [239]. The observed discrepancy between the literature and measured K_{oc} values was explained as an artefact due to the difficulties with measuring free concentrations of highly hydrophobical substances in other studies [239]. Indeed, any overestimation of freely dissolved concentrations would cause an underestimation of the true K_{oc} values [235]. In order to perform a similar comparison between field- and lab-derived K_{oc} values, we calculated log $K_{oc,field}$ from our data as follows:

$$logK_{oc,field} = log\left(\frac{C_{sol} \cdot f_{oc,sol}}{C_{diss}}\right) \tag{5.5}$$

The difference between log $K_{oc.field}$ and log K_{oc} was on average 1.76 ± 0.63 for sediment and 1.78 ± 0.62 for SPM (see Figures C.5 and C.6 in Appendix C section C.6 for a graphical representation), which is similar to the findings of Hawthorne et al. [237]. As such, differences between field- and lab-derived K_{oc} values are a likely explanation for the poor performance of the equilibrium model for sediment and SPM. Also, like for the whole water concentrations, the underestimation of PAH and PCB concentrations in solids may be due to the presence of

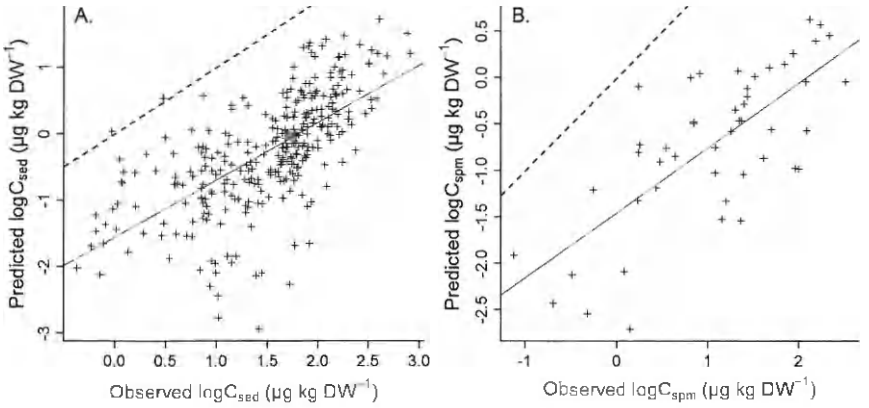

Figure 5.3: Predicted vs. observed concentrations of PAHs and PCBs in solids. The solid line represents the correlation between the measured and predicted concentrations, the dashed line represents the 1:1 relationship. A. Sediment (PCC = 0.66, N = 322, $p < 0.001$). B. SPM (PCC = 0.73, N = 48, $p < 0.001$).

BC. However, it is questionable whether the inclusion of BC could eliminate the observed underestimation by the equilibrium model for solids. Indeed, as mentioned above K_{BC} is on average a factor of 37 higher than K_{oc}. Given that BC generally represents only a small fraction of the total organic carbon content of solids (e.g. the total organic carbon in marine sediments can contain between 15 and 30% of BC [240]), including BC would not be sufficient to resolve the underestimation of PAH and PCB concentrations in sediments and SPM.

Table 5.1: 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. The last row represents the model bias. N: number of data points; MB: model bias.

	Sediment (N=322)	SPM (N=48)
Factor 2	0.9	0
Factor 5	3.7	2.1
Factor 10	9	12.5
Factor 100	67.1	68.8
Factor 1000	96.3	96.3
MB	0.02	0.02

5.3.3 Concentrations in biota

The equilibrium approach explored in this paper performed better for biota than 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% (shrimp) were accurate within a factor of 5 (Table 5.2). The MB indicated a general tendency of the equilibrium model to overestimate the data with a factor of up to 3.65, which was also apparent when assessing the model fit (see Figure C.7in Appendix C section C.7). When further exploring the data, it becomes apparent that this bias is mainly caused by compounds with a $\log K_{ow} \geq 6.5$, and that this bias becomes stronger as $\log K_{ow}$ further increases (Figure 5.4). To a certain extent 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, at least for the higher trophic levels [241, 242]. As such, one would expect a modelling approach entirely based on equilibrium partitioning theory to underestimate rather than overestimate body burdens as hydrophobicity increases. Possibly, this seemingly contradictory observation can be explained by (1) the relatively low trophic level of the species considered here, (2) biotransformation, or (3) the loss of a linear relationship between $\log K_{ow}$ and uptake of contaminants in organisms. The latter has been described in literature extensively [241, 243–246]. 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 faeces of very hydrophobic compounds [246, 247]. Other scientists claim it to be an artefact arising from so-called third phase effects and nonequilibrium conditions occurring during laboratory bioconcentration measurements [248]. The former occur when the aqueous concentration of an organic chemical – necessary to calculate its bioconcentration factor – is overestimated due to the fraction of the chemical sorbed to dissolved organic matter. While the real reason for the deviation observed in this study cannot be deduced from our data, a few comments can be made. As the concentration data derived from passive sampling represent the freely dissolved and thus by definition the bioavailable fractions of the contaminants, a reduced bioavailability cannot be the reason for the discrepancy we observe for biota. What remains is the possibility of a lower uptake rate combined with growth dilution and/or a higher excretion rate of the highly hydrophobic contaminants, thereby causing a divergence from equilibrium conditions. This may be further explored by using more advanced modelling techniques (e.g. the use of food web models) rather than using a simple equilibrium model.

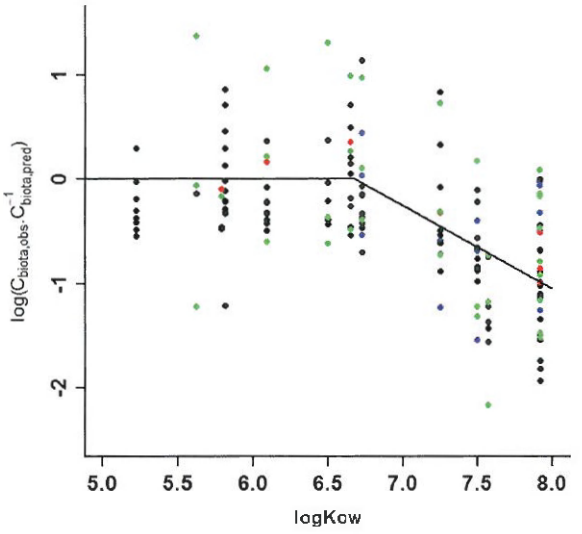

Figure 5.4: The logarithm of the ratio of observed and predicted concentration data in biota vs. logK_{ow}. Blue: shrimp, black: mussels, red: fish tissue, green: fish liver.

Table 5.2: 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. The last row represents the model bias. N: number of data points; MB: model bias.

	Shrimp (N=15)	Mussel tissue (N=101)	Fish liver (N=34)	Fish muscle (N=10)
Factor 2	20	30.7	26.5	30
Factor 5	73.3	68.3	47.1	70
Factor 10	73.3	82.2	67.6	90
Factor 100	100	100	97.1	100
MB	3.65	2.66	2.08	1.30

5.4 Conclusions

This chapter shows that a passive sampling approach combined with a simple equilibrium partitioning model can function as a baseline or 'null' model to predict contaminant concentrations in different environmental matrices. Deviations between these prediction and observations can be used to hypothesize which processes contribute to the environmental behaviour of these chemicals. When extended with these processes, e.g. using more advanced modelling approaches such as pharmacokinetic models or foodweb models, passive samplers can be a cost-effective way to estimate true integrated exposure profiles to organic pollutants in the marine environment.

6

Risk assessment of PAHs and PCBs in the Belgian marine environment using passive sampling data.

Redrafted from: Claessens, M., De Laender, F., Monteyne, E., Roose, P., Janssen, C.R. Risk assessment of PAHs and PCBs in the Belgian marine environment using passive sampling data. Chemosphere, to be submitted.

Abstract

Obtaining reliable information on aquatic contaminant concentrations for use in environmental and human health risk assessments is costly and time-consuming. For this reason, passive sampling devices (PSDs) have been increasingly used as an alternative for conventional monitoring practices. PSDs allow the derivation of freely dissolved, time-weighted average concentrations of contaminants, have much lower detection limits and are easier to analyze.

Concentration data derived from passive sampling have also been used to estimate contaminant concentrations in other aquatic compartments (e.g. sediment and biota) through equilibrium modelling. In this study, an environmental and human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) was conducted on the basis of such modelled concentration data and was subsequently compared to the results of a conventional risk assessment.

In general, the risks predicted on the basis of passive sampling data were well able to cover the range of risks observed by conventional methods for all environmental compartments that were included in the assessment. Some differences were observed for the aquatic compartment and for biota. If these differences are taken into account, the proposed methodology may already be suitable as a rapid, cheap methodology to monitor risks of contaminants at sites of good chemical status.

6.1 Introduction

The aim of this chapter is to perform an environmental and human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) based on concentration data derived from passive sampling in the Belgian marine environment. Up to now, such an exercise has been done in only a few studies elsewhere in which the risk assessments were limited to the water phase [176, 249, 250] and/or the risks to human health via the consumption of contaminated seafood [249, 251]. In Chapter 5, equilibrium modelling based on freely dissolved concentrations of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) as derived from passive sampling was used to predict the concentrations of these compounds in other matrices (i.e. whole-water, sediment, suspended particulate matter (SPM) and aquatic organisms). In that chapter, the concentrations of these compounds were consistently underpredicted in sediment and SPM. However, there was a good correlation between predicted and measured concentrations and the model bias could be explained by the use of organic carbon-water partitioning coefficients (K_{oc}) that were derived in laboratory studies. Such lab-derived K_{oc} values have been found to underestimate the partitioning of hydrophobic micropollutants to sediments in the field (e.g. Hawthorne et al. [237]). The prediction of aquatic concentrations and concentrations in biota were generally good, although model biases were observed for both compartments above certain hydrophobicity thresholds.

The objective of the current study is to use the concentrations predicted with the models in Chapter 5 to perform an environmental and human health risk assessment of PAHs and PCBs in the Belgian marine environment. The results of this risk assessment will be compared with a conventional risk assessment based on measured concentration data from spot sampling campaigns. The model for calculating sediment concentrations will first be improved by adjusting the K_{oc} values to better reflect partitioning of PAHs and PCBs to sediment under field conditions.

6.2 Materials and methods

6.2.1 Distribution modelling

The modelling in this study builds further on the results obtained in Chapter 5. In that chapter, we used freely dissolved concentrations of PAHs and PCBs derived through passive sampling in simple equilibrium models to estimate concentrations of these compounds in sediment and biota as well as their total concentration in water (i.e. including the amount sorbed to dissolved organic carbon (DOC) and particulate organic carbon (POC)).

In Chapter 5 we observed a consistent underprediction of PAH and PCB concentrations in sediment and SPM. However, a strong correlation between the observed and predicted data for these compartments was noted. This was potentially due to the use of K_{oc} values derived using standard methodologies which can give rise to artefacts and inconsistencies when considering partitioning to organic carbon in field sediments and SPM. As such, in this study we re-ran the equilibrium model for solids by adjusting the log K_{oc} values obtained from literature using a correction factor (CF):

$$logK_{oc,field} = logK_{oc} + CF \tag{6.1}$$

A separate CF was applied for PAHs and PCBs based on data from Hawthorne et al. [237] and Achman et al. [252], respectively. In these studies, measured log $K_{oc,field}$ values were determined for PAHs and PCBs in marine and estuarine sediments, respectively. The average difference between log K_{oc} and the log $K_{oc,field}$ values from Hawthorne et al. [237] and Achman et al. [252] were determined for both groups of compounds (see Appendix D section D.2), yielding CFs of 1.51±0.31 and 0.93±0.27 for PAHs and PCBs, respectively.

To study the performance of the corrected model, the model bias (MB) was calculated as:

$$MB = 10^{\frac{\sum_{i}^{n} log\frac{Predicted(i)}{Observed(i)}}{n}} \tag{6.2}$$

with Predicted(i) as the concentration predicted by the model in the respective matrix at a given location and time, Observed(i) as the corresponding measured concentration and n as the number of observations. Additionally, as in Chapter 5, the accuracy of the model predictions was assessed by calculating the percentage of predicted data falling within a certain factor of the observed data.

All modelling was performed using the software R [231].

6.2.2 Environmental risk assessment

The quality criteria used to assess the risks of chemicals in the water column were annual average environmental quality standards (AA-EQS) for the marine environment and were extracted from a decision of the Flemish government [253] and from European Directive 2008/105/EC [254] (Table 6.1). The risk assessment for this compartment consisted of the calculation of the risk characterisation ratio for water (RCR_{water}) as follows:

$$RCR_{water} = \frac{EC_{ww}}{AA-EQS} \tag{6.3}$$

in which EC_{ww} is the whole water concentration of PAHs or PCBs, which was derived either by conventional sampling and chemical analysis or by passive sampling and subsequent modelling (see Chapter 5). An RCR_{water} greater or equal to unity indicates a risk is present for the organisms living in the marine water compartment.

Table 6.1: Environmental quality criteria of PAHs and PCBs for the water and sediment compartment. QC: quality criterion, AA-EQS: annual average environmental quality standard, PNEC: predicted no-effect concentration, LL-EAC: lower limit environmental assessment criterion, p: provisional.

	Water			Sediment		
Compound	Value (µg L^{-1})	QC	Reference	Value (µg kg^{-1} DW)	QC	Reference
PAHs						
1-methylnaphthalene	–	–	–	–	–	–
2-methylphenanthrene	–	–	–	–	–	–
2,6-dimethylnaphthalene	–	–	–	–	–	–
Acenaphthene	0.06	AA-EQS	[253]	0.16	PNEC	[255]
Acenaphthylene	4	AA-EQS	[253]	–	–	–
Anthracene	0.1	AA-EQS	[254]	50	LL-EAC	[256]
Benzo(a)anthracene	0.3	AA-EQS	[253]	100 (p)	LL-EAC	[256]
Benzo(a)pyrene	0.05	AA-EQS	[254]	100 (p)	LL-EAC	[256]
Benzo(b)fluoranthene	sum = 0.03	AA-EQS	[254]	140	PNEC	[255]
Benzo(k)fluoranthene				140	PNEC	[255]
Benzo(ghi)perylene	sum = 0.002	AA-EQS	[254]	84	PNEC	[255]
Indeno(1,2,3-cd)pyrene				63	PNEC	[255]
Benzo(e)pyrene	–	–	–			
Chrysene	1	AA-EQS	[253]	100 (p)	LL-EAC	[256]
Dibenz(a,h)anthracene	0.5	AA-EQS	[253]	–	–	–
Fluoranthene	0.1	AA-EQS	[254]	500 (p)	LL-EAC	[256]
Fluorene	2	AA-EQS	[253]	–	–	–
Naphthalene	1.2	AA-EQS	[254]	50	LL-EAC	[256]
Perylene	–	–	–	–	–	–
Phenanthrene	0.1	AA-EQS	[253]	100	LL-EAC	[256]
Pyrene	0.04	AA-EQS	[253]	50 (p)	LL-EAC	[256]
PCBs						
sum7PCBs	0.002	AA-EQS	[253]	1 (p)	LL-EAC	[256]

The quality criteria used for the risk assessment of PAHs and PCBs present in sediments were PNEC values taken from a risk assessment report on coal tar pitch [255] and lower limit environmental assessment criteria (LL-EAC) from OSPAR [256] (Table 6.1). Similar as for the water compartment, a risk characterisation ratio ($RCR_{sediment}$) was calculated:

$$RCR_{sediment} = \frac{EC_{sediment}}{PNEC or LL-EAC} \tag{6.4}$$

in which $EC_{sediment}$ is the environmental concentration of PAHs or PCBs in sediment as derived by conventional sampling and chemical analysis or by equilibrium partitioning modelling using passive sampling data. An $RCR_{sediment}$ greater or equal to unity indicates a risk is present for the organisms living in or in close contact with the sediment compartment.

For biota, LL-EAC values were taken from OSPAR [256] for both PAHs and PCBs (Table 6.2). The risk characterisation ratio for biota (RCR_{biota}) was calculated as:

$$RCR_{biota} = \frac{EC_{biota}}{LL-EAC} \tag{6.5}$$

in which EC_{biota} is the environmental concentration of PAHs or PCBs in biota (bivalves) as derived by conventional sampling and chemical analysis or by equilibrium partitioning modelling using passive sampling data. An RCR_{biota} greater or equal to unity indicates a risk is present.

Table 6.2: Environmental quality criteria of PAHs and PCBs for biota (secondary poisoning). QC: quality criterion, LL-EAC: lower limit environmental assessment criterion. LL-EAC: lower limit environmental assessment criterion, p: provisional, f: firm.

	Bivalves		
Compound	Value (µg kg^{-1} DW)	QC	Reference
PAHs			
1-methylnaphthalene	–	–	–
1-methylphenanthrene	–	–	–
2,6-dimethylnaphthalene	–	–	–
Acenaphthene	–	–	–
Acenaphthylene	–	–	–
Anthracene	5 (p)	LL-EAC	[256]
Benzo(a)anthracene	–	–	–
Benzo(a)pyrene	5000 (p)	LL-EAC	[256]
Benzo(b)fluoranthene	–	–	–
Benzo(k)fluoranthene	–	–	–
Benzo(ghi)perylene	–	–	–
Indeno(1,2,3-cd)pyrene	–	–	–
Benzo(e)pyrene	–	–	–
Chrysene	–	–	–
Dibenz(a,h)anthracene	–	–	–
Fluoranthene	100 (p)	LL-EAC	[256]
Fluorene	–	–	–
Naphthalene	500 (p)	LL-EAC	[256]
Perylene	–	–	–
Phenanthrene	5000 (p)	LL-EAC	[256]
Pyrene	100 (p)	LL-EAC	[256]
PCBs			
sum7PCBs	5 (f)	LL-EAC	[256]

6.2.3 Human health risk assessment

The risks of PCBs for human health through the consumption of seafood were assessed using seafood consumption data from the EFSA Comprehensive European Food Consumption Database [257] (Table 6.3). This database contains food consumption data for a variety of age groups, but for this study the highest seafood intake for adults (see bold values in Table 6.3) was used to obtain a realistic worst case risk estimation. The health quality criteria used for this assessment (Table 6.4) were taken from WHO [258]. For PAHs, the maximum level (ML) of benzo(a)pyrene – used in Europe as a reference compound for the other PAHs in the context of human health – allowed in different types of seafood was obtained from EU Regulation 1881/2006 [259] (Table 6.4).

The measured concentration data of PCBs in biota (no measured data were available for benzo(a)pyrene), which were originally expressed on a dry weight basis, were converted to a wet weight basis by assuming an average water content

of 77% for all biota [260–262]. The concentration data in biota obtained through passive sampling were originally expressed on a lipid-normalized dry weight basis. These data were converted to a wet weight basis using measured lipid contents for the different organisms and a water content of 77%. Subsequently, the RCR for human health (RCR_{HH}) was calculated as follows for PCBs:

$$RCR_{HH,PCB} = \frac{C_{biota,PCB} \cdot CR}{TDI} \tag{6.6}$$

with $C_{biota,PCB}$ as the PCB concentration in biota (expressed in µg PCB per g wet tissue weight) determined by conventional or passive sampling, CR as the consumption rate (expressed in g wet tissue per kg body weight (BW) per day, see Table 6.3) and TDI as the total daily intake (expressed in µg wet tissue per kg BW per day, see Table 6.4). $RCR_{HH,PCB}$ was calculated and assessed separately for shrimp, bivalves and fish.

Table 6.3: Selected consumption rates (CR) of seafood for Belgian adults expressed as g of food per kg body weight per day. P5 and P99 are the 5th and 99th percentile, respectively. The values in bold were used for the human health risk assessment. SD: standard deviation. Source: EFSA [257].

	P5	Mean	P99	SD
Water molluscs	0.11	0.72	**2.36**	0.52
Crustaceans	0.03	0.41	**2.43**	0.46
Fish meat	0.09	0.80	**3.32**	0.68

For benzo(a)pyrene, RCR_{HH} was calculated as follows:

$$RCR_{HH,BaP} = \frac{C_{biota,BaP}}{ML} \tag{6.7}$$

with $C_{biota,BaP}$ as the benzo(a)pyrene concentration in biota (expressed in µg benzo(a)pyrene per kg wet tissue weight) and ML as the maximum level in biotic tissue (expressed in µg benzo(a)pyrene per kg wet tissue weight, see Table 6.4).

Table 6.4: Quality criteria of benzo(a)pyrene and PCBs for the assessment of human health risks through the consumption of seafood.

Compound	Value	Unit	QC	Reference
	2	µg per kg WW	ML (fish)	[259]
Benzo(a)pyrene	10	µg per kg WW	ML (bivalve)	[259]
	5	µg per kg WW	ML (crustacean)	[259]
Sum(PCBs)	0.02	µg per kg BW per day	TDI	[258]

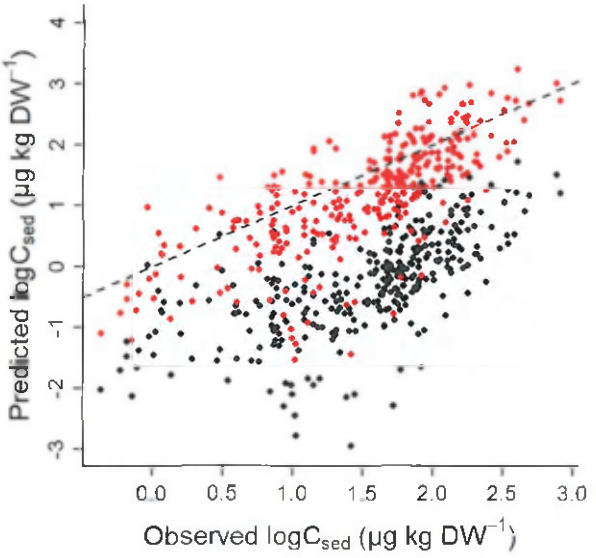

Figure 6.1: Observed versus predicted concentrations of PAHs and PCBs in sediment. The dashed line represents the 1:1 relationship. Black dots represent the model results based on conventional log K_{oc} values, red dots represent the model results based on log $K_{oc,field}$ values.

6.3 Results

6.3.1 Model performance for sediment

The equilibrium model originally performed very poorly for sediment, generally underpredicting the PAH and PCB concentrations by a factor of 50 (see Chapter 5). Using $K_{oc,field}$ the model performance increased significantly (Figure 6.1), limiting the underprediction to a factor of 2 as indicated by the MB of 0.47. Roughly 90% of the predicted values were accurate within an order of magnitude and approximately 76% was accurate within a factor of 5. This is a considerable improvement over the original model that could only predict 9% of the concentrations within an order of magnitude of the measured data (see Chapter 5). As such, the results from this improved model were used in the subsequent risk assessment.

6.3.2 Environmental risk assessment

In the presentation of the results and the following discussion, RCRs based on measured concentration data will be referred to as a 'measured RCRs' while RCR based on the modelled concentration data will be referred to as a 'predicted RCRs'.

6.3.2.1 Aquatic compartment

In the harbours, measured RCRs showed that quality criteria for the aquatic compartment were exceeded for acenaphthene, the sum of benzo(b)fluoranthene and benzo(k)fluoranthene, the sum of benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene and the sum of PCBs (Figure 6.2). Generally, risks were observed in less than 5 % of the cases, except for the sum of benzo(b)fluoranthene and benzo(k)fluoranthene for which a risk was observed in 33 % of the cases (Table D.3). These results are similar to the findings of Ghekiere et al. [263], in which a similar environmental risk assessment was performed on the same data. In general though, the risk assessment performed in Ghekiere et al. [263] – which was only based on conventional sampling data – was less detailed and no ranges of RCRs were reported.

Predicted RCRs for the harbours showed risks for acenaphthene and the sum of PCBs in 4 % and 14 % of the cases, respectively. At sea stations, risks were observed for the sum of benzo(b)fluoranthene and benzo(k)fluoranthene in 15 % of the cases and these risks were only apparent when using measured data.

At the offshore stations, there seemed to be a higher discrepancy between measured and predicted RCRs – the former in general being higher than the latter – compared to within the harbours (Figures 6.2 and 6.3). This is most likely due to the fact that at passive sampling station RV no spot samples were taken. Indeed, as for the offshore stations the spot sampling data from stations S01 and W01–W06 were used while passive sampling was only conducted at stations A2 (once) and RV (3 times), a certain discrepancy could be expected due to spatial variation. This makes the offshore region perhaps less suitable for making a good comparison between the two risk assessment approaches.

A number of substances seemed to exhibit a larger difference between measured and predicted RCRs than others. Indeed, the predicted RCRs in harbours of benzo(a)pyrene, the sum of benzo(b)fluoranthene and benzo(k)fluoranthene and the sum of benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene differed a factor of 13, 19 and 16, respectively, from their measured RCRs. For the other contaminants, this difference was on average only a factor of 2. For the offshore stations, a higher apparent discrepancy between measured and predicted RCRs was also observed for benzo(a)anthracene and dibenz(a,h)anthracene. This may be due to the abovementioned spatial variation. However, it is also important to note that the lower limit of the RCRs is determined by the detection limit of the analytical methodology. This detection limit is much higher for conventional chemical analysis of water samples than for passive sampling, generally being in the lower ng L^{-1} range for the former and in the lower pg L^{-1} range for the latter. Therefore, when

environmental contaminants are present below the detection limit of conventional techniques but can be detected by passive sampling, the lowest measured RCR will by definition be higher than the lowest predicted RCR.

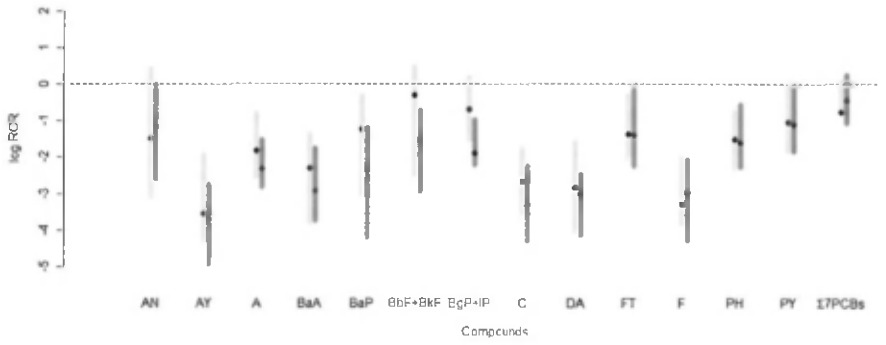

Figure 6.2: Comparison of measured and predicted RCR values of PAHs and PCBs in water of the harbours. The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respectively. Black dots represent the median values. The dashed line represents the threshold (i.e. RCR = 1) above which the contaminants pose a risk towards the environment. 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)fluoranthene, BgP: benzho(g,h,i)perylene, IP: indeno(1,2,3-cd)pyrene, $\sum$7PCBs: sum of PCB congeners 28, 52, 101, 118, 138, 153 and 180.

6.3.2.2 Sediment

The ranges of measured and predicted RCRs of PCBs and PAHs obtained for sediment are graphically depicted in Figures 6.4 and 6.5 for harbours and offshore stations, respectively (see Table D.4 for the underlying data). The median predicted RCRs were within a factor of 3 of the median measured RCRs in 81% of the cases. In the harbours, risks were observed for all compounds and these risks were apparent in both predicted and measured RCRs. Figure 6.4 shows that the predicted RCRs were able to cover the magnitude, range and variability of the measured RCRs very well.

At offshore stations, measured RCRs indicated risks for acenaphthene, indeno(1,2,3-cd)pyrene, pyrene and the sum of PCBs. With the exception of indeno-(1,2,3-cd)pyrene, predicted RCRs indicated risks for these compounds as well. It was apparent that at the offshore stations the overlap of measured and predicted RCRs was not as good as for the harbour stations (Figure 6.5). Like for the aquatic

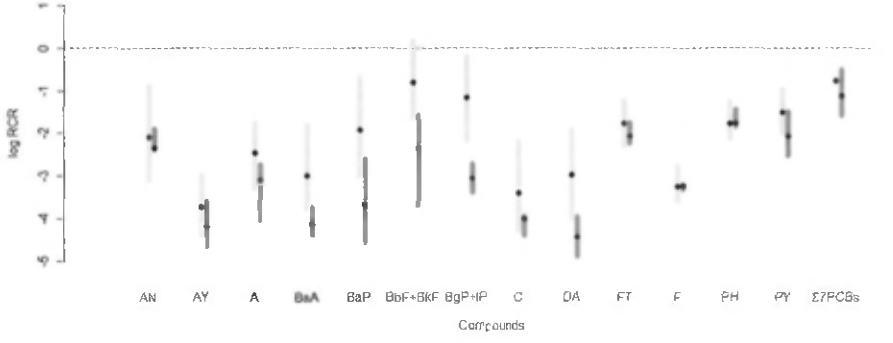

Figure 6.3: Comparison of measured and predicted RCR values of PAHs and PCBs in offshore water. The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respectively. Black dots represent the median values. The dashed line represents the threshold (i.e. RCR = 1) above which the contaminants pose a risk towards the environment. For an explanation of the abbreviations, see Figure 6.2.

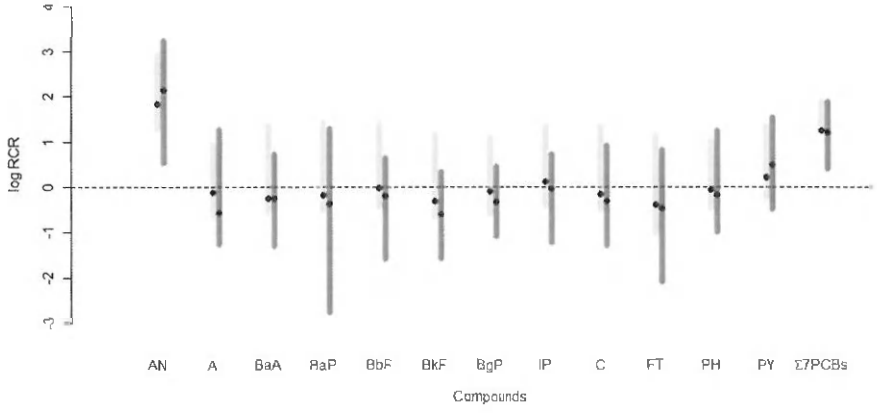

Figure 6.4: Comparison of measured and predicted RCR values of PAHs and PCBs in sediment of the harbours. The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respectively. Black dots represent the median values. The dashed line represents the threshold (i.e. RCR = 1) above which the contaminants pose a risk towards the environment. For an explanation of the abbreviations, see Figure 6.2.

compartment, this is probably due to the fact that for passive sampling station RV no sediment samples were available for direct comparison. Indeed, the measured RCRs shown in Figure 6.5 represent the ranges of RCRs of all 7 offshore stations. Since the offshore passive sampling data originate from 3 sampling events at station RV and 1 sampling at station A2 (Table D.1), again a certain discrepancy may be expected.

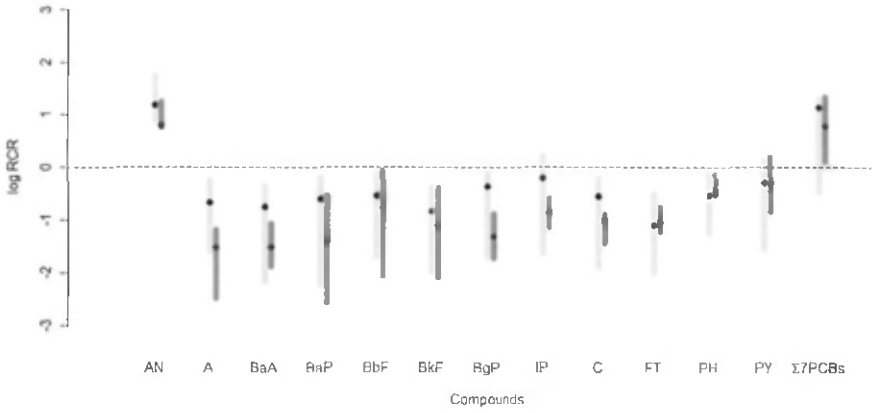

Figure 6.5: Comparison of measured and predicted RCR values of PAHs and PCBs in offshore sediments. The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respectively. Black dots represent the median values. The dashed line represents the threshold (i.e. RCR = 1) above which the contaminants pose a risk towards the environment. For an explanation of the abbreviations, see Figure 6.2.

6.3.2.3 Aquatic organisms

For contaminants in bivalves a comparison between measured and predicted RCRs was only possible for pyrene and the sum of 7 PCBs as only for these compounds measured concentrations in mussel tissue were available (Figure 6.6). Of the 5 PAHs for which LL-EAC values were available (Table 6.2), passive sampling data only indicated risks for fluoranthene in 4% of the cases in the harbour area. Measured RCRs indicated risks for pyrene in 31% of the cases in the harbours. These risks were not reflected by the passive sampling data, which led to a median predicted RCR of almost a factor of 6 lower than the measured RCR. A similar difference between measured and predicted RCRs for pyrene was observed in the offshore area.

At offshore stations, no risks were observed for any of the PAHs. For the sum of PCBs risks were observed in 100% of the cases at harbour and offshore stations and this was indicated both by measured and predicted RCRs. The quality criterion

for PCBs was exceeded by a factor of up to 83 and 20 as indicated by passive sampling data from the harbours and offshore stations, respectively (see Appendix D.3). Median predicted and measured RCRs for the sum of PCBs differed little.

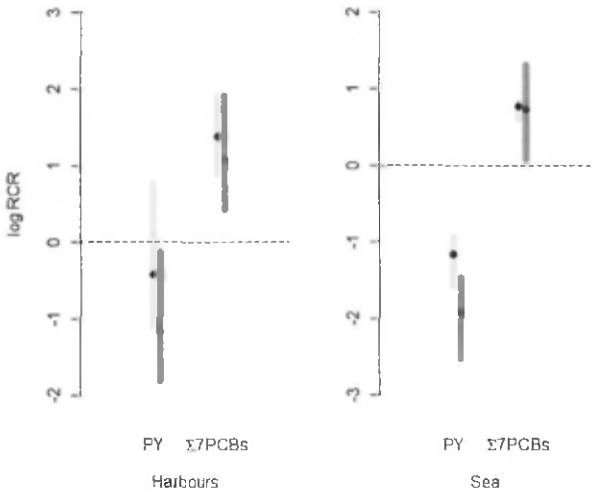

Figure 6.6: Comparison of measured and predicted RCR values for pyrene and PCBs in bivalves (Mytilus edulis). The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respectively. Black dots represent the median values. The dashed line represents the threshold (i.e. RCR = 1) above which the contaminants pose a risk towards the environment. PY: pyrene, ∑7PCBs: sum of 7 PCBs.

6.3.2.4 Human secondary poisoning

The ranges of measured and predicted RCRs of PCBs for secondary poisoning in humans due to consumption of seafood are presented in Figure 6.7 (see Appendix D.3 for the numerical data). As for benzo(a)pyrene no measured data in organism tissue were available, a comparison between measured and predicted RCRs could only be made for the sum of PCBs. In the harbours, risks were observed in 100% of the cases for potential consumption of bivalves contaminated with PCBs as reflected by the predicted RCRs. The measured RCRs indicated risks in 93 % of the cases. Even at offshore stations, risks were apparent in 56% and 100% percent of the cases as reflected by measured and predicted RCRs for bivalves, respectively. Risks due to the consumption of fish contaminated with PCBs were apparent in 75% and 80% of the cases as reflected by predicted and measured RCRs for offshore stations, respectively. The quality criterion for fish was exceeded by a factor of up to 20 as indicated by passive sampling data. The predicted RCRs for PCBs

indicated higher risks than the measured RCRs, the medians of the former being roughly a factor of 4 higher in the 4 cases that allowed comparison.

For benzo(a)pyrene the predicted RCRs indicated few risks in the harbours for the consumption of shrimp and fish, both organisms that are generally not caught in harbour environments. Indeed, the predicted RCRs from the harbour stations are mostly theoretical as in principle seafood for human consumption never originates from this area. An exception is station OO1, where aquaculture activities are present. For bivalves, the predicted RCR for benzo(a)pyrene did not exceed unity in the harbour area.

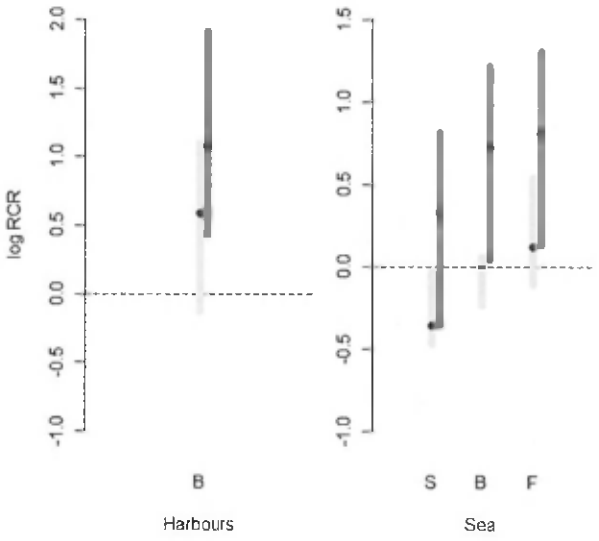

Figure 6.7: Comparison of measured and predicted RCR values for human health of PCBs in biota. The light grey and dark grey bars represent the range of RCRs (from minimum to maximum value) based on measured and predicted concentrations, respectively. The dashed line represents the threshold (i.e. RCR = 1) above which the contaminants pose a risk towards human health. Black dots represent the median values. S: shrimp, B: bivalves, F: fish.

6.4 Discussion

In general, both similarities and differences were observed between the measured and predicted RCRs of PAHs and PCBs for the different marine compartments included in this study. Measured and predicted RCRs were the most similar for sediments. This could be expected since concentrations of contaminants in sediment exhibit a much lower variability than aqueous concentrations. Thus, the former

represent – like the contaminant concentrations in passive samplers – concentrations averaged over time. The overlap between measured and predicted RCRs for sediment was better in the harbours than in the offshore area. This is most likely because in the harbours, unlike in the offshore area, the passive sampling stations were in close proximity to the conventional sampling stations. Overall, the method proposed in this study was able to cover the range of risks in the sediment compartment observed by conventional methods very well.

The differences between measured and predicted RCRs were most pronounced for the whole water concentrations, at least for a number of the PAHs. This may be due to the fact that contaminant concentrations in grab samples of water represent only a single moment in time and are the most susceptible to temporal variation compared to the other compartments. As the contaminant concentrations in passive samplers also represent averaged levels, a perfect match between the two methods is unlikely. However, as we observed in Chapter 5, the equilibrium model exhibited an increasing underprediction of the whole water concentrations with increasing log K_{oc} values of the contaminants. Given that in the harbour area the ranges of predicted and measured RCRs were very similar for a majority of the compounds, the discrepancy observed for a number of the PAHs may be due to difficulties to calculate partitioning towards DOC and/or POC.

For biota, only little analytical data were available on tissue concentrations of PAHs making an in-depth comparison difficult both for aquatic organisms and for secondary poisoning of humans. Predicted RCRs for total PCBs (i.e. for human health risk assessment) seemed to be higher than measured RCRs while the overlap was better when the sum of 7 PCBs was used (i.e. for the risks towards aquatic organisms). As shown in Chapter 5, there was an increasing overprediction of contaminant concentrations in biota with increasing hydrophobicity, which explains this observation. As we discussed in that study, this was potentially due to elimination pathways that are not present in passive samplers causing the contaminant concentrations in these surrogates to be higher than in biota. Allan et al. [251] – who compared contaminant concentrations in PSDs directly to quality criteria – argued that the fact that PSDs do not metabolize contaminants is an advantage as it thus provides a more complete exposure potential. However, for the purpose of a risk assessment, metabolism of compounds is a relevant elimination pathway as are growth dilution and excretion. Hence, such physiological processes should be accurately represented by any biomimetic technique used in environmental risk assessment. Of course, elimination mechanisms differ between different organisms and therefore the use of passive samplers does offer a neutral baseline method that can be used to predict concentrations in a multitude of organisms. If more accurate tissue concentrations are needed, more complex models (than the equilibrium

model used here) should be applied (see Chapter 5). The proposed methodology offers a number of advantages over conventional monitoring techniques. The use of PSDs can be standardized across studies and using them as a surrogate for biota eliminates the difficulties associated with sampling and analyzing organisms [251]. The same can be said for sediment and whole water contaminant concentrations, as the chemical analysis of these matrices is also more difficult than analyzing passive sampler extracts.

6.5 Conclusions

Overall, the results of the risk assessments obtained in this chapter demonstrate that passive sampling can offer an alternative for conventional monitoring techniques as it is able to cover well the range of risks observed by conventional methods for all environmental compartments that were included, with only few exceptions for some specific compounds. As the methodology stands, it may already be suitable to monitor locations where no (more) risks from pollutants are present. Taking into account the necessary uncertainty, passive sampling and subsequent (equilibrium) modelling could in such cases be used as a rapid, cheap methodology to ascertain that contaminant concentrations remain sufficiently low for risks to remain absent. As such, the methodology in its current form could be used as one of the first steps in a tiered risk assessment approach, whereby the subsequent tiers could involve more complex modelling of the passive sampling data and whereby the more expensive conventional sampling and chemical analytical techniques can be used as the final tier if potential risks are observed in all the lower tiers.

7

General conclusions

7.1 Introduction

The work presented in this doctoral thesis focussed on the use of passive samplers as a tool in environmental risk assessment and was an integral part of the INRAM[1] project [188]. The main aim of the INRAM project was to conduct an integrated risk assessment of micropollutants in the Belgian marine environment using passive sampling as a novel, central tool (see Chapter 1.4, Figure 1.8). As such, the use of passive samplers was studied (1) as a device to first collect environmental contaminant mixtures through *in situ* passive sampling and subsequently perform ecotoxicity testing on these mixtures through passive dosing, and (2) as a surrogate phase for water, solids (sediment and SPM) and biota to conduct – through equilibrium modelling – an environmental exposure and subsequent risk assessment. This is schematically represented in Figure 7.1.

In the following sections, the main conclusions obtained in this thesis (highlighted in bold font) are summarised and discussed. The shortcomings of conventional monitoring and hazard assessment approaches that were listed in Chapter 1.3, will specifically be addressed (highlighted in underline font) and suggestions for future research will be given.

7.2 Assessing the hazard of environmental mixtures through passive dosing

In conventional aquatic toxicity tests, toxicants are dissolved in the water phase (sometimes with the aid of a carrier solvent). For certain chemicals (e.g. hydrophobic substances) however, it is <u>difficult to maintain the exposure levels throughout the test due to substance losses via adsorption to test vessel walls or organic matter, degradation, bio-uptake or volatilisation</u>. While certain experimental setups can be used to address this issue – such as flow-through experiments (see Chapter 1.2.2) – they are generally much more complex and higher in costs. Moreover, for ecotoxicity studies with unicellular algae the renewal of the test medium is technically not feasible.

In **Chapter 3** a passive dosing methodology to administer and maintain constant concentrations of hydrophobic chemicals in ecotoxicological test medium, was developed and fine-tuned. The passive dosing experiments confirmed that PDMS sheets loaded with hydrophobic chemicals (i.e. PAHs) were **able to achieve stable concentrations of these substances in the test medium for at least 72 hours**. The test setup used in this study – i.e. adding 3.5 g of contaminated PDMS sheets to 50 mL of test medium – is most suitable for substances with a log $K_d \geq 2$.

[1] INRAM stands for Integrated Risk Assessment and Monitoring of micropollutants along the Belgian coastal zone and is a project sponsored by the Belgian Federal Science Policy Office (BELSPO).

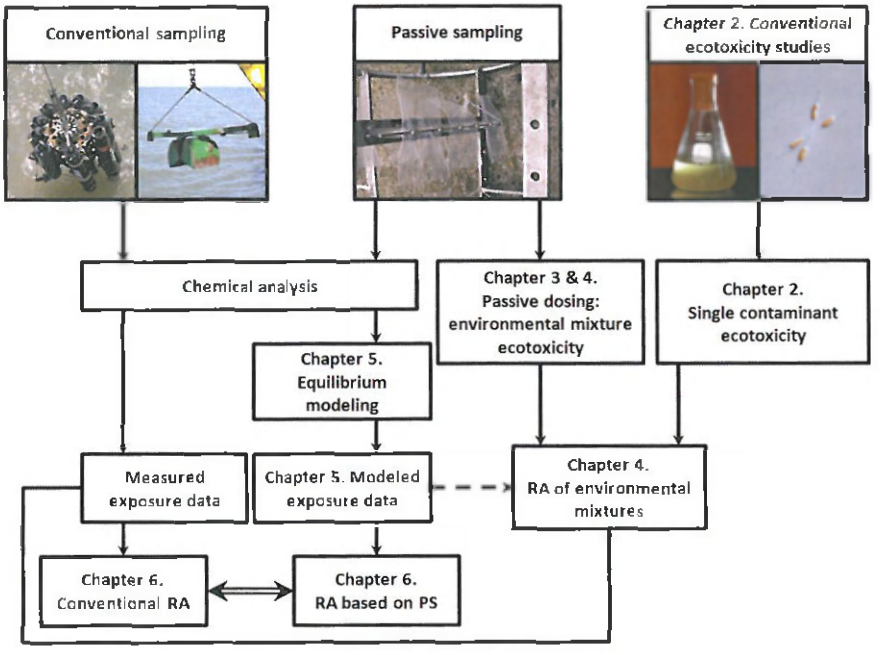

Figure 7.1: Schematic overview of the work included in this thesis. Topics covered in a specific chapter are indicated by the chapter number. Source of the picture of P. tricornutum (top right): [264].

Less hydrophobic substances would become depleted in the samplers by more than 10% as was determined in a modelling exercise.

While the passive dosing technique is not new and has been applied using different kinds of materials (e.g. [60, 62, 155, 161]) including PDMS (as coatings applied on test vessels or in rods) [61, 63, 66, 156, 163, 166], it has not been performed with the form of PDMS used in this study (i.e. prefabricated sheets). The latter was chosen for the work in this thesis as it is a type that can be easily used for *in situ* passive sampling and was thus suitable for the work described in **Chapter 4**. In that chapter, the passive dosing technique was applied using PDMS sheets that had been previously deployed in the field. As such, the environmental contaminant mixtures collected in the field through passive sampling were recreated in the laboratory test medium through passive dosing. This allowed exposure of the test organism *P. tricornutum* to environmentally relevant contaminant mixtures under controlled laboratory conditions. The main advantage of this technique, is that **the concentrations of chemicals in the mixture represent the average concentrations in the environment during the time of passive sampling**. This is in strong contrast with whole sample bioassays in which organisms are exposed to a grab sample of water. In that case, the mixture offers only a snapshot of

constantly varying contaminant concentrations. It must be noted, however, that **for very hydrophobic compounds (log K_d>4) equilibrium was not reached after the passive sampling period** and as such these chemicals are underrepresented in the mixtures recreated in the laboratory through passive dosing. Moreover – as mentioned above – the more hydrophylic compounds (log K_d<2) are likely to become depleted significantly from the PDMS phase during passive dosing, which also leads to lower concentrations in the test medium compared to the average environmental levels.

Most of the mixtures generated through passive dosing did not cause adverse effects on the growth of *P. tricornutum*. In some cases however, a growth inhibition of up to 100 % was observed. These severe effects could not be explained based on the substances that had been identified and measured in grab samples collected at the sampling sites or in the passive sampler extracts. Indeed, the high costs associated with chemical analysis drive the need to make a selection of chemicals to be studied. Monitoring campaigns hence typically focus on only a limited number of chemicals that are of potential concern (such as the priority substances of the Water Framework Directive).

→ **Suggestions and recommendations for further research**

In order to help elucidate the severe adverse effects on the growth of *P. tricornutum* caused by some of the environmental mixtures (**Chapter 4**), new analytical techniques such as **high-resolution full scan analysis** should be further explored. Such techniques for which Time-of-Flight and Orbitrap instruments are very suitable, allow the simultaneous analysis of a virtually unlimited number of chemicals, both targeted and untargeted [30, 210, 211]. Wille et al. [30] for example, identified 3 untargeted chemicals (2 pharmaceuticals and a pesticide) in passive sampler extracts using Orbitrap MS. As such these techniques may help to identify the chemicals responsible for the growth inhibition observed in *P. tricornutum*. Nonetheless, there are still limitations to this methodology [30] and as such it is recommended to **further refine these techniques so their full potential can be utilised**. In further studies, it is recommended to include naturally occurring substances as well, as for example marine toxins – which can have adverse effects on a wide range of species, including humans – have been detected in passive samplers as well. Thus, such substances may be (in part) responsible for the adverse effects on the growth of *P. tricornutum* as observed in this thesis.

Further opportunities for improvement lie in the passive sampling and dosing techniques. Indeed, in the passive dosing experiments conducted in **Chapter 4** the concentrations of very hydrophobic compounds (log K_d>4) as well as more hydrophylic compounds (log K_d<2) were likely to be lower in the test medium

than in the environment. The **use of multiple passive sampling materials** that differ in their affinity for the target chemicals as well as in their chemical uptake and release characteristics, can help to overcome these problems.

7.3 Passive samplers as a surrogate for other aquatic compartments

Conventional monitoring studies are expensive due to high costs of (1) using research vessels and (2) the chemical analysis of grab samples, which is often labour intensive and costly due to difficulties associated with the extraction of chemicals from complex matrices like sediment and biota. Moreover, the contaminant concentrations measured in one or a few grab samples do not accurately reflect the general pollution levels (see Chapter 1.2.1). Consequently, a high sampling frequency is required to perform an adequate exposure assessment. Passive sampling can help reduce the monitoring costs as it reflects average contaminant concentrations. Of course, *in situ* passive sampling of sediments is practically difficult – especially at locations where the sea is (very) deep – and is impossible for biota. As such, in **Chapter 5** concentrations of PAHs and PCBs in water, SPM, sediment and organisms were predicted based on aquatic passive sampling data and equilibrium modelling. The concentrations of these chemicals were predicted well for water and organisms, although some biases that seemed related to hydrophobicity were observed. For sediment and SPM, the equilibrium initially underpredicted the measured concentrations consistently which was attributed to the use of lab-derived K_{oc} data to model partitioning of the PAHs and PCBs to solids. By correcting the K_{oc} data based on knowledge of *in situ* partitioning of these substances to sediments (Chapter 6), this underestimation was significantly reduced. As such, this approach allowed to obtain reliable estimates of the concentrations of PAHs and PCBs in water, solids and biota. As environmental concentrations derived from passive sampling reflect average contaminant concentrations, **passive sampling campaigns can be conducted less frequently than conventional monitoring campaigns, thus significantly lowering the costs**. Moreover, **the extraction of passive samplers and the subsequent chemical analysis of the extracts is much less complex than extracting and analyzing whole water, sediment and biota samples.** Additionally, as passive samplers accumulate contaminants, they **allow for lower concentrations to be detected** and are thus capable of providing a **more complete picture of chemical pollution**.

In Chapter 6 the results of this modelling exercise were further used to perform an environmental and human health risk assessment of PAHs and PCBs present in the Belgian marine environment. Overall, the risks observed via passive sam-

pling data covered the ranges of risks predicted using conventional monitoring data well. However, for the aquatic compartment and for secondary poisoning of humans, passive sampling data indicated much lower risks for some compounds than the conventionally obtained concentration data. The same was true for pyrene in biota, of which the concentrations seems to be under-predicted. These findings need to be taken into account if passive sampling is used for risk assessments in this manner. Nonetheless, this risk assessment approach could for example be used at locations where safe levels of all measured pollutants have been demonstrated. When appropriately taking into account the uncertainties described above, **a risk assessment solely based on passive sampling and subsequent equilibrium modelling results can be used to ascertain that pollutants levels remain low**. As mentioned above, such an approach could greatly reduce the need for conventional environmental monitoring and the associated costs.

→ **Suggestions and recommendations for further research**

Further studies on predicting the concentrations of chemicals in water, sediment and biota may focus on (1) the refinement of the equilibrium model or (2) the use of other, potentially more appropriate models. Regarding the estimation of contaminant concentrations in whole water samples, sediment and SPM, a refinement of the modelling could be achieved by further elucidating the observed discrepancy between partitioning to organic carbon according to experimental K_{oc} values and the partitioning observed *in situ*. This observation may be due to the presence of other carbon forms – such as black carbon – that have higher sorptive capacities for the target substances. This has already been the subject of many studies (e.g. Accardi-Dey and Gschwend [265]) in which it was demonstrated that the inclusion of other carbon forms can help explain sorption of chemicals to sediments in field conditions. However, this has not yet led to a unified approach that can be used to more reliably assess this process for a diverse set of sediments. In this regard, it may be helpful to focus on the general applicability of the correction that was applied to K_{oc} values in this thesis. Indeed, the correction factors used to adapt the K_{oc} values of PAHs and PCBs were derived from field studies from other locations (i.e. rural and urban waterways [237] and lake Michigan [252], both located in North America), which has nonetheless lead to a reliable model for predicting concentrations of these compounds in Belgian marine sediments. As such, the applied correction may prove to be sufficient for other locations as well, which could render the need for more mechanistic modelling obsolete. Of course, if the *in situ* adsorption characteristics of solids do vary too much spatially, there may also be the need to investigate if similar variations occur on a temporal basis. Indeed, the adsorption characteristics of sediment and SPM may vary in time as well and the extent of this variation should in that case be established.

For the prediction of concentrations in organisms, it can be explored if the use

of bioconcentration factors (BCFs) can be helpful to address the observed biases. Another option would be to use more complex models that take into account all the uptake and elimination pathways of substances.

Of course, it must always be kept in mind that a certain deviation between conventionally measured contaminant levels in grab samples and the concentrations derived through passive sampling and modelling will exist. Indeed, it can not be expected that contaminant levels in immobile passive samplers fully reflect the contamination levels in mobile organisms and dynamic sediments, which are both subject to transport during a passive sampling campaign. Unless detailed knowledge on the transport processes is available, it is difficult to establish the general, local pollution levels based on a limited number of grab samples of sediments or organisms. As such, while passive samplers may lead to apparent exposure levels that are somewhat different than those derived by conventional sampling, the former may actually be more representative of the general local pollution levels than the latter. Passive sampling may therefore have the potential to entirely change the existing monitoring approaches. To this end, extensive studies comparing both approaches will be necessary.

A

Supporting data for Chapter 2

A.1 Literature ecotoxicity data

Table A.1: Literature ecotoxicity data for the pharmaceuticals studied in Chapter 2.

Compound	Species	Endpoint	Concentration ($mg\ L^{-1}$)	Reference
	Phytoplankton			
Salicylic acid	*S. subspicatus*	72h, growth inhibition, EC50	>100	[100]
	P. subcapitata	72h, growth inhibition, EC50	22.7	[266]
	Zooplankton			
Salicylic acid	*D. magna*	48h, immobility, EC50	111.7	[114]
	D. magna	48h, immobility, EC50	118	[100]
	D. magna	48h, immobility, EC50	870	[112]
	D. magna	immobility, EC50	143	[94]
	D. magna	48h, immobility, EC50	1945	[110]
	D. longispina	48h, immobility, EC50	1148	[110]
	D. magna	24h, immobility, EC50	230	[96]
	D. longispina	21d, reproduction, NOEC	5.6	[110]
	D. magna	21d, reproduction, NOEC	>10	[110]
	Fish			
Salicylic acid	*D. rerio*	48h, mortality, LC50	24.6	[103]
	B. rerio	48h, mortality, LC50	37	[100]
	L. idus	mortality, LC50	90	[94]
	Phytoplankton			
Paracetamol	*S. subspicatus*	72h, growth inhibition, EC50	134	[100]
	P. subcapitata	96h, growth inhibition, EC50	2300	[267]
	P. subcapitata	96h, growth inhibition, NOEC	550	[267]
	Zooplankton			
Paracetamol	*D. magna*	24h, immobility, EC50	55.5	[97]
	A. salina	24h, mortality, LC50	577.5	[97]
	S. proboscideus	24h, mortality, LC50	29.6	[97]
	D. magna	48h, mortality, LC50	20.1	[114]
	D. magna	48h, immobility, EC50	50	[100]
	D. magna	48h, immobility, EC50	30.1	[117]
	D. magna	48h, immobility, EC50	9.2	[95]
	D. pulex	24h, immobility, EC50	136	[99]
	D. magna	48h, immobility, EC50	17	[267]
	Fish			
Paracetamol	*P. promelas*	96h, mortality, LC50	814	[98]
	B. rerio	48h, mortality, LC50	378	[100]
	O. latipes	96h, mortality, LC50	800	[267]
	Phytoplankton			
Carbamazepine	*D. subspicatus*	72h, growth inhibition, EC50	74	[106]
	C. meneghiniana	96h, growth inhibition, EC50	31.6	[91]
	S. leopolensis	96h, growth inhibition, EC50	33.6	[91]
	C. vulgaris	48h, growth inhibition, EC50	36.6	[105]
	P. subcapitata	96h, growth inhibition, EC50	64	[267]
	C. meneghiniana	96h, growth inhibition, NOEC	10	[91]
	S. leopolensis	96h, growth inhibition, NOEC	17.5	[91]
	P. subcapitata	96h, growth inhibition, NOEC	6.4	[267]
	Zooplankton			
Carbamazepine	*H. azteca*	10d, mortality, LC50	9.9	[119]
	D. magna	48h, immobility, EC50	13.8	[268]
	C. dubia	48h, mortality, LC50	77.7	[91]
	D. magna	48h, mortality, LC50	111	[114]
	D. magna	48h, immobility, EC50	97.8	[105]

Continued on next page

Table A.1 – continued from previous page

Compound	Species	Endpoint	Concentration ($mg\ L^{-1}$)	Reference
	D. magna	96h, immobility, EC50	76.3	[117]
	D. magna	48h, immobility, EC50	55	[267]
	C. dubia	7d, reproduction, NOEC	0.025	[91]
	Fish			
Carbamazepine	*O. latipes*	96h, mortality, LC50	35.4	[117]
	O. latipes	96h, mortality, LC50	45.87	[120]
	O. latipes	96h, mortality, LC50	61.5	[125]
	O. latipes	96h, mortality, LC50	20	[267]
	D. rerio	10d, mortality, NOEC	25	[91]
	Phytoplankton			
Atenolol	*D. subspicatus*	72h, growth inhibition, EC50	620	[107]
	P. subcapitata	72h, growth inhibition, EC50	190	[122]
	P. subcapitata	72h, growth inhibition, EC50	143	[122]
	P. subcapitata	96h, growth inhibition, EC50	110	[267]
	P. subcapitata	72h, growth inhibition, NOEC	128.8	[126]
	P. subcapitata	96h, growth inhibition, NOEC	10	[267]
	Zooplankton			
Atenolol	*D. magna*	48h, immobility, EC50	313	[107]
	D. magna	48h, immobility, EC50	1450	[122]
	D. magna	48h, immobility, EC50	755	[122]
	C. dubia	48h, immobility, EC50	33.4	[111]
	D. magna	48h, immobility, EC50	200	[109]
	D. magna	48h, immobility, EC50	180	[267]
	D. magna	21d, reproduction, NOEC	8.9	[126]
	Fish			
Atenolol	*O. latipes*	96h, mortality, LC50	>100	[120]
	O. latipes	96h, mortality, LC50	1800	[267]
	P. promelas	28d, growth, NOEC	3.2	[128]
	Phytoplankton			
Propranolol	*D. subspicatus*	72h, growth inhibition, EC50	5.8	[106]
	D. subspicatus	72h, growth inhibition, EC50	0.7	[107]
	C. meneghiniana	96h, growth inhibition, EC50	0.244	[91]
	S. leopolensis	96h, growth inhibition, EC50	0.668	[91]
	P. subcapitata	96h, growth inhibition, EC50	7.4	[91]
	P. subcapitata	72h, growth inhibition, EC50	0.77	[124]
	P. subcapitata	96h, growth inhibition, EC50	0.66	[267]
	C. meneghiniana	96h, growth inhibition, NOEC	0.094	[91]
	P. subcapitata	96h, growth inhibition, NOEC	5	[91]
	S. leopolensis	96h, growth inhibition, NOEC	0.35	[91]
	P. subcapitata	96h, growth inhibition, NOEC	0.1	[267]
	Zooplankton			
Propranolol	*S. proboscideus*	24h, mortality, LC50	1.87	[97]
	D. magna	24h, immobility, EC50	15.8	[97]
	A. salina	24h, mortality, LC50	407	[97]
	D. magna	48h, immobility, EC50	7.5	[106]
	D. magna	48h, immobility, EC50	7.7	[107]
	D. magna	48h, mortality, LC50	2.75	[91]
	C. dubia	48h, mortality, LC50	1.51	[91]
	C. dubia	48h, immobility, EC50	1.4	[111]
	C. dubia	48h, mortality, LC50	0.8	[104]
	D. magna	48h, mortality, LC50	1.6	[104]
	H. azteca	48h, mortality, LC50	29.8	[104]
	T. platyrus	24h, mortality, LC50	10.31	[120]
	D. pulex	24h, immobility, EC50	3.833	[99]
	D. magna	24h, immobility, EC50	2.7	[99]
	D. magna	48h, immobility, EC50	1.4	[115]
	D. magna	48h, immobility, EC50	1.57	[115]

Continued on next page

Table A.1 – continued from previous page

Compound	Species	Endpoint	Concentration (mg L^{-1})	Reference
	D. magna	48h, immobility, EC50	1.67	[115]
	D. magna	48h, immobility, EC50	0.46	[267]
	C. dubia	7d, reproduction, NOEC	0.009	[91]
	C. dubia	7d, reproduction, NOEC	0.125	[104]
	H. azteca	27d, reproduction, NOEC	0.001	[104]
	Fish			
Propranolol	*O. latipes*	48h, mortality, LC50	24.3	[104]
	O. latipes	96h, mortality, LC50	11.4	[120]
	P. promelas	48h, mortality, LC50	1.42	[115]
	P. promelas	48h, mortality, LC50	1.69	[115]
	P. promelas	48h, mortality, LC50	1.21	[115]
	O. latipes	96h, mortality, LC50	9	[267]
	D. rerio	10d, mortality, NOEC	2	[91]
	O. latipes	14d, growth, NOEC	0.1	[104]
	O. latipes	28d, egg production, NOEC	<0.0005	[104]
	Phytoplankton			
Bezafibrate	*Anabaena sp.*	24h, growth inhibition, EC50	7.62	[127]
	Zooplankton			
Bezafibrate	*D. magna*	48h, immobility, EC50	30.3	[114]
	T. platyurus	24h, mortality, LC50	39.69	[116]
	D. magna	24h, immobility, EC50	100.08	[116]
	C. dubia	24h, immobility, EC50	75.79	[116]
	D. magna	48h, immobility, EC50	240.4	[127]
	C. dubia	7d, reproduction, NOEC	0.023	[116]
	Phytoplankton			
Trimethoprim	*S. carpricornutum*	72h, growth inhibition, EC50	80.3	[108]
	S. carpricornutum	growth inhibition, EC50	110	[102]
	S. carpricornutum	growth inhibition, EC50	130	[101]
	M. aeruginosa	7d, growth inhibition, EC50	112	[101]
	R. salina	growth inhibition, EC50	16	[101]
	P. subcapitata	72h, growth inhibition, EC50	40	[93]
	S. carpricornutum	72h, growth inhibition, NOEC	25.5	[108]
	P. subcapitata	72h, growth inhibition, NOEC	16	[93]
	Zooplankton			
Trimethoprim	*D. magna*	96h, immobility, EC50	120.7	[117]
	D. magna	48h, immobility, EC50	149	[123]
	D. magna	48h, immobility, EC50	92	[121]
	M. macropaca	48h, immobility, EC50	54.8	[121]
	D. magna	48h, immobility, EC50	167.4	[118]
	D. magna	48h, immobility, EC50	123	[102]
	D. magna	21d, reproduction, NOEC	6	[121]
	Fish			
Trimethoprim	*O. latipes*	96h, mortality, LC50	>100	[117]

A.2 Individual contribution of components to total mixture toxicity

The individual contribution of pharmaceutical i (IC_i) to the cumulative risk posed by the entire mixtures ($RCR_{MEC/PNEC}$), was calculated as:

$$IC_i = \frac{RCR_i}{RCR_{MEC/PNEC}} \cdot 100 \quad \text{(A.1)}$$

The results are summarized in Table A.2.

Table A.2: Median contribution of the individual pharmaceuticals (IC in %, median calculated over the different sampling periods) to the cumulative risk posed by the mixtures expressed as the total toxicity $RCR_{MEC/PNEC}$. SAL: salicylic acid; CAR: carbamazepine; ATE: atenolol; PRO: propranolol; BEZ: bezafibrate; TRI: trimethoprim.

	SAL	CAR	ATE	PRO	BEZ	TRI
W01	7.5	20.6	0.3	21.2	46.1	0.4
W02	7.4	5.5	0.4	24.9	54.1	0.5
W03	2.9	5.8	0.4	28.2	61.2	0.6
W04	3.3	16.9	0.4	24.1	52.3	0.5
W05	2.3	5.7	0.4	28.6	62.3	0.6
W06	2.7	5.7	0.4	28.5	62.0	0.6
Offshore median	**3.1**	**5.8**	**0.4**	**26.5**	**57.6**	**0.6**
NP1	0.6	22.9	0.1	60.9	15.5	0.1
NP2	0.6	14.4	0.2	57.0	25.3	0.2
NP3	1.1	5.8	0.4	29.2	60.9	0.6
OO1	6.2	20.8	0.1	43.8	31.5	0.2
OO2	1.5	9.0	0.1	61.6	25.5	0.1
OO3	1.3	9.0	0.1	57.2	33.2	0.1
OO4	0.5	9.5	0.1	53.2	36.2	0.1
ZB1	1.8	20.0	0.3	37.3	40.5	0.4
ZB2	5.1	19.1	0.3	30.2	42.6	0.4
ZB3	4.3	21.5	0.2	36.1	33.5	0.3
ZB4	2.3	19.1	0.2	44.1	34.5	0.3
Harbour median	**1.5**	**19.1**	**0.2**	**44.1**	**33.5**	**0.2**
S01	3.3	19.4	0.3	30.5	41.5	0.4
S22	0.6	17.1	0.1	76.0	5.3	0.0
Scheldt median	**1.9**	**18.3**	**0.2**	**53.2**	**23.4**	**0.2**
Overall median	**2.3**	**16.9**	**0.3**	**36.1**	**40.5**	**0.4**

B

Supporting data for Chapter 4

B.1 QSAR for PAHs

A QSAR model was developed to estimate 72h algal growth inhibition values of PAHs. The data used for this exercise are listed in Table B.1. The EC50 values of the PAHs were related to their log K_{ow} values through linear regression analysis. This is graphically represented in Figure B.1. The resulting QSAR relationship is (N = 5, R^2 = 0.87):

$$LogEC50 = -1.24 log K_{ow} + 8.71 \quad \text{(B.1)}$$

Table B.1: Log K_{ow} values and ecotoxicity of PAHs used for the development of a QSAR model. The EC50 values were generated for this thesis using the 72h algal growth inhibition study with P. tricornutum. The EC50 value of fluoranthene was taken from Wang et al. [192].

Compound	Log K_{ow}	Log EC50
Fluorene	4.18	3.60
Phenanthrene	4.46	3.07
Anthracene	4.45	3.24
Pyrene	5.22	2.55
Fluoranthene	5.16	2.01

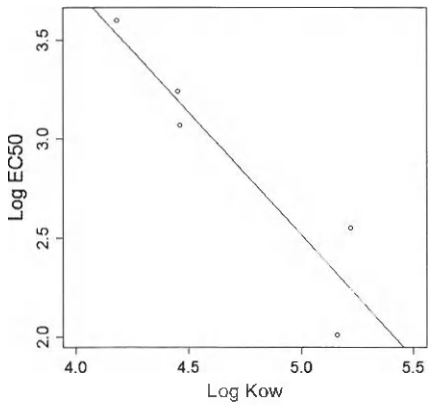

Figure B.1: Scatter plot of log K_{ow} versus log EC50 of the PAHs listed in Table B.1. The line represents the regression relationship (log EC50 = -1.24 log K_{ow} + 8.71; N = 5, R^2 = 0.87, p = 0.022).

B.2 Equilibrium in the passive sampling campaigns

In Monteyne et al. [176], passive samplers spiked with performance reference compounds (PRCs) were deployed in parallel with the samplers used in our study. The PRCs allow the determination of the time-weighted average (TWA) concentrations of compounds for which the uptake was still in the linear phase (i.e. for which equilibrium had not been reached after the sampling period, see Monteyne et al. [176] for further details). The time needed to reach equilibrium becomes longer with increasing hydrophobicity. Chemical analysis of the PRCs pointed out that equilibrium had been reached for compounds with a log K_d of 4.1 on average. Indeed, when considering all passive sampling campaigns together, the PRCs with a log K_d of 4.1 had dissipated from the samplers for 90%. Table B.2 presents, for all the separate passive sampling campaigns, the log K_d values of PRCs that had dissipated from the samplers for 10, 50 and 90%. It is important to note that these log K_d values do not necessarily represent the precise log K_d of one of the PRCs. This is because these data were calculated from a nonlinear curve fitted to the full PRC dissipation dataset. More details on this can be found in Monteyne et al. [176].

Table B.2: Estimated log K_d values at which a certain percentage of the performance reference compounds (PRCs) had dissipated from the passive samplers after the respective sampling periods. For example: at the end of the sampling campaign of April-July 2008, 90% of the PRCs with a log K_d of 4.7 had dissipated from the samplers that were deployed at station NP1.

Sampling period	PRC depletion	NP1	NP2	OO1	OO2	OO3	ZB2	ZB3	RV
Apr–Jul 2008	90%	4.7	4.6	3.8	4.0	4.0	4.1	4.0	4.8
	50%	5.2	5.1	4.4	4.5	4.5	4.6	4.5	5.3
	10%	6.0	5.9	5.2	5.3	5.3	5.4	5.3	6.1
Oct–Dec 2008	90%	–	3.8	–	4.1	–	3.7	3.6	–
	50%	–	4.3	–	4.6	–	4.2	4.1	–
	10%	–	5.2	–	5.4	–	5.1	4.9	–
Aug–Oct 2009	90%	–	3.9	–	4.0	–	4.1	3.7	4.1
	50%	–	4.4	–	4.5	–	4.6	4.2	4.7
	10%	–	5.2	–	5.3	–	5.4	5.0	5.5

C

Supporting data for Chapter 5

C.1 Sampling scheme

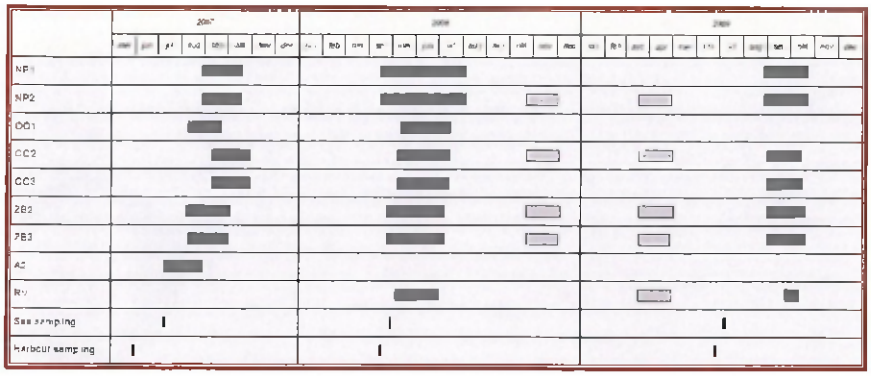

Figure C.1: Full sampling scheme. The dark grey bars represent the passive sampling campaigns conducted as part of the integrated campaigns that included conventional monitoring. The light grey bars represent the passive sampling campaigns conducted in parallel with caged mussels as part of a biomarker experiment. The last two rows represent the periods in which the conventional monitoring campaigns were conducted at open sea and in the harbours, respectively.

C.2 Overview of available analytical data

Table C.1 presents an overview of the data availability on PAH and PCB concentrations in the passive samplers and in the grab samples of water, sediment, SPM and biota. These data were used in the equilibrium modelling conducted in this study. Detailed concentration data are available via the INRAM project results (www.vliz.be/projects/inram) and have partly been published in scientific literature [176].

Table C.1: Overview of the substances that were measured in the different matrices during the different sampling events. The years indicated as 07, 08 and 09 indicate the 3 conventional sampling campaigns. B08 and B09 indicate the passive sampling events that were conducted in parallel with caged mussels as part of a biomarker experiment. The PCBs, when measured, consisted of the congeners listed in 2.1. x: measured, –: not measured.

	Water			SPM			Sediment			Shrimp			Mussels		Fish tissue			Fish liver			PS			
	07	08	09	07	08	09	07	08	09	07	08	09	B08	B09	07	08	09	07	08	09	07	08	B08	09	B09
acenaphthylene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
acenaphthene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
2,3,5-trimethylnaphthalene	x	x	x	–	–	–	x	–	–	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
fluorene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
phenanthrene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
anthracene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
pyrene	x	x	x	x	x	x	x	x	x	x	–	–	x	x	–	–	–	x	–	–	x	x	x	x	x
1-methylphenanthrene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
fluoranthene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
benzo(a)anthracene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
chrysene	x	x	x	x	x	x	x	x	x	x	–	–	x	x	–	–	–	x	–	–	x	x	x	x	x
benzo(e)pyrene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
benzo(a)pyrene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
benzo(ghi)perylene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	–	–	x	x	x
indeno(1,2,3-cd)pyrene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	–	–	x	x	x
benzo(b)fluoranthene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
benzo(k)fluoranthene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	x	x	x	x	x
dibenz(a,h)anthracene	x	x	x	x	x	x	x	x	x	x	–	–	–	–	–	–	–	x	–	–	–	–	x	x	x
PCBs	x	x	–	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

C.3 Physicochemical data of the study compounds

The physicochemical data of the PAHs and PCBs included in this study are given in Table C.2. For some of the compounds, no experimental log K_{ow} and log K_{oc} values were available. For the latter, values were estimated using KOCWIN from EPI-Suite [269]. For log K_{ow}, missing values were determined by relating this parameter to molecular weight (MW). For PAHs, this yielded the following regression relationship (N = 13, $R^2 = 0.98$, $p < 0.001$):

$$logK_{ow} = 0.022 \cdot MW + 0.67 \quad \text{(C.1)}$$

For PCBs, the regression relationship (N = 10, $R^2 = 0.94$, $p < 0.001$) was:

$$logK_{ow} = 0.017 \cdot MW + 1.12 \quad \text{(C.2)}$$

Both regressions are graphically depicted in Figure C.2.

Table C.2: Physicochemical characteristics of the PAHs and PCBs used in this study. Log K_{ow} values were taken from Hansch et al. [80], values in italics were calculated based on molecular weight using Equations C.1 and C.2. Log K_{oc} values were taken from Schüürmann et al. [270] and Meylan et al. [271], values in italics were estimated using KOCWIN from EPI-Suite [269]. Log K_{DOC} values were calculated as log K_{DOC} = 0.97 log K_{ow} - 1.27 [235]. Log K_{POC} values were calculated as log K_{POC} = 0.899 log K_{ow} + 0.328 [234]. Log K_{BC} values of PAHs are from Hawthorne et al. [272], values in italics were estimated by regression analysis with molecular weight (N = 20, R^2 = 0.953, log K_{BC} = 0.02 MW + 1.87). Log K_{BC} values of PCBs were calculated according to the equation by Di Paolo et al. (log K_{BC} = 0.928 log K_{ow} + 0.080) [273]. Log K_d values were taken from Smedes et al [177]. MW: molecular weight.

Compound	MW (g mol^{-1})	log K_{ow}	log K_{oc}	log K_{DOC}	log K_{POC}	log K_{BC}	log K_d
acenaphthylene	152.2	3.94	3.75	2.55	3.87	5.56	3.25
acenaphthene	154.21	3.92	3.59	2.53	3.85	4.90	3.62
2,3,5-trimethylnaphthalene	170.26	*4.35*	*4.00*	2.95	4.24	*5.54*	4.01
fluorene	166.22	4.18	3.7	2.78	4.09	5.19	3.79
phenanthrene	178.24	4.46	4.35	3.06	4.34	5.61	4.11
anthracene	178.24	4.45	4.31	3.05	4.33	6.12	4.21
pyrene	202.26	4.88	4.9	3.46	4.72	6.26	4.67
1-methylphenanthrene	192.26	*5.08*	*4.42*	3.66	4.89	*6.03*	4.27
fluoranthene	202.26	5.16	4.8	3.74	4.97	6.25	4.62
benzo(a)anthracene	228.3	5.79	5.3	4.35	5.53	7.07	5.31
chrysene	228.3	*5.73*	*5.15*	4.29	5.48	7.03	5.25
benzo(e)pyrene	252.32	*6.12*	5.68	4.67	5.83	7.18	5.64
benzo(a)pyrene	252.32	5.97	5.95	4.52	5.70	7.22	5.7
benzo(ghi)perylene	273.34	6.63	*6.02*	5.16	6.29	7.75	6.03
indeno(1,2,3-cd)pyrene	276.34	*6.64*	6.2	5.17	6.30	8.27	6.06
benzo(b)fluoranthene	252.32	*6.12*	*5.90*	4.67	5.83	7.28	5.74
benzo(k)fluoranthene	252.32	*6.12*	*5.77*	4.67	5.83	7.28	5.74
dibenz(a,h)anthracene	278.36	6.5	6.22	5.04	6.17	7.40	6.24
PCB18	257.55	5.6	4.4	4.16	5.36	5.23	5.23
PCB28	257.55	5.62	4.63	4.18	5.38	5.30	5.53
PCB31	257.55	5.79	4.68	4.35	5.53	5.36	5.49
PCB44	291.99	5.81	4.9	4.37	5.55	5.47	5.82
PCB52	291.99	6.09	4.85	4.64	5.80	5.73	5.81
PCB101	326.44	6.5	5.22	5.04	6.17	6.39	6.28
PCB105	326.44	6.65	5.12	5.18	6.31	6.52	6.43
PCB118	326.44	*6.73*	5.11	5.26	6.38	6.69	6.42
PCB138	360.88	7.25	5.93	5.76	6.85	6.98	6.77
PCB153	360.88	7.5	5.58	6.01	7.07	7.27	6.72
PCB156	360.88	7.57	5.33	6.07	7.13	7.13	6.73
PCB170	395.33	7.92	5.55	6.41	7.45	7.45	7.11
PCB180	395.33	7.92	5.54	6.41	7.45	7.40	7.00
PCB187	395.33	7.92	5.54	6.41	7.45	7.31	6.88

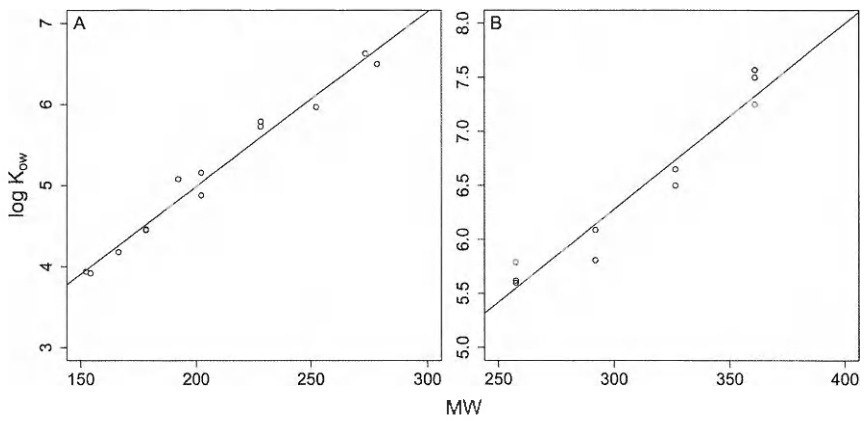

Figure C.2: Log Kow of PAHs and PCBs plotted against molecular weight (MW). The solid lines represent the linear regression relationship. A. PAHs (N = 13, R^2 = 0.98, $p < 0.001$). B. PCBs (N = 10, R^2 = 0.94, $p < 0.001$).

C.4 Log K_{oc} versus log K_{DOC}, log K_{POC} and log K_{BC}

Figure C.3 presents the results of linear regressions relating log K_{oc} to the partitioning coefficients for DOC, POC and BC. This resulted in the following regression relationships:

$$logK_{DOC} = 1.19logK_{oc} - 1.55 \tag{C.3}$$

$$logK_{POC} = 1.11logK_{oc} - 0.07 \tag{C.4}$$

$$logK_{BC} = 1.10logK_{oc} - 0.93 \tag{C.5}$$

The slopes of these equations were all moderately higher than 1, indicating an increasing difference between the partitioning coefficients with increasing hydrophobicity.

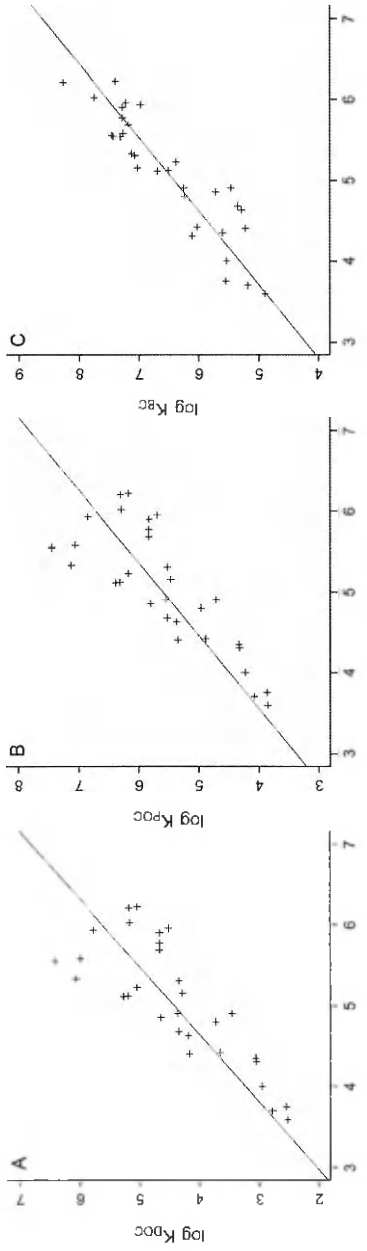

Figure C.3: Results of linear regression analysis relating log K_{oc} to (A) log K_{DOC} ($N = 32$, $R^2 = 0.613$, $p < 0.001$, log K_{DOC} = 1.19 log K_{oc} -1.55), (B) log K_{POC} ($N = 32$, $R^2 = 0.613$, $p < 0.001$, log K_{POC} = 1.11 log K_{oc} - 0.07) and (C) log K_{BC} ($N = 32$, $R^2 = 0.816$, $p < 0.001$, log K_{BC} = 1.10 log K_{oc} - 0.93).

C.5 Modelling whole water concentrations using K_{DOC} and K_{POC} values

In an attempt to improve the performance of the equilibrium model for predicting whole water concentrations of PAHs and PCBs in water from passive sampling-derived freely dissolved concentrations, K_{oc} values were substituted by DOC-water partitioning coefficients K_{DOC} and POC-water partitioning coefficients K_{POC}. The log Kdoc values were calculated with the equation for surface waters from Burkhard et al. [235]:

$$logK_{DOC} = 0.97 \cdot logK_{ow} - 1.27 \tag{C.6}$$

Log K_{POC} values were calculated according to the equation from Lüers and Hulscher [234]:

$$logK_{POC} = 0.899 \cdot logK_{ow} + 0.328 \tag{C.7}$$

As such, whole water concentrations were calculated as follows:

$$C_{ww} = C_{diss}(1 + K_{DOC} \cdot [DOC] + K_{POC} \cdot [POC]) \tag{C.8}$$

Using this equilibrium model, 48.9% and 65.9% of the predicted data were accurate within a factor of 5 and within an order of magnitude of the measured data, respectively. The model bias was 0.29 in this case. As such, the original model in which the standard K_{oc} values were used, performed better than this adapted model.

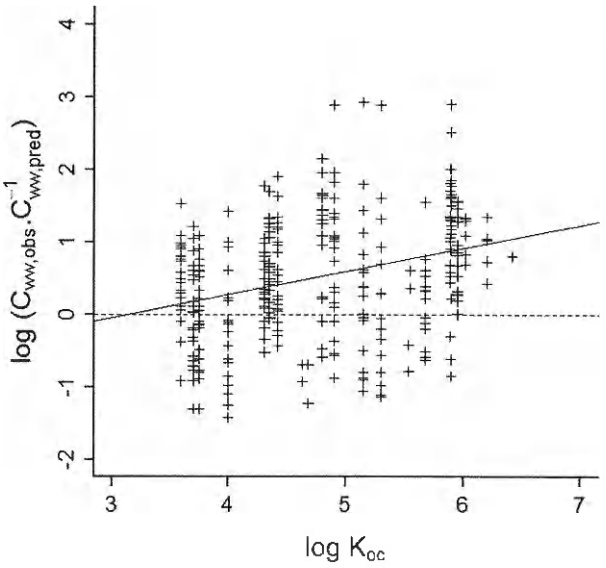

Figure C.4: Equilibrium model results for the prediction of whole water concentrations of PAHs and PCBs based on freely dissolved concentrations of these compounds as derived from passive sampling. The solid line represents the regression line (log $(c_{ww,obs}.c^{-1}_{ww,pred})$ *= 0.32 log* K_{oc} *- 1.00, N = 324,* R^2 *= 0.098,* $p < 0.001$*). Where log* $(c_{ww,obs}.c^{-1}_{ww,pred})$ *equals zero (dashed line), observed and predicted data are equal.*

C.6 Lab- versus field-derived K_{OC} data

Figures C.5 and C.6 show a comparison between literature values for log K_{oc} and the *in situ* log K_{oc} values derived in this study for sediment and SPM, respectively. The latter were derived as:

$$logK_{oc,field} = log\left(\frac{C_{sol} \cdot f_{oc,sol}}{C_{diss}}\right) \tag{C.9}$$

with log $K_{oc,field}$ as the *in situ* log K_{oc} values, C_{sol} as the concentration of contaminants in solids (i.e. either sediment or SPM), $f_{oc,sol}$ as the fraction of organic carbon in the solids and C_{diss} as the freely dissolved contaminant concentration derived by passive sampling. The difference between log $K_{oc,field}$ and log K_{oc} was on average 1.76 $\pm$ 0.63 for sediment and 1.78 $\pm$ 0.62 for SPM.

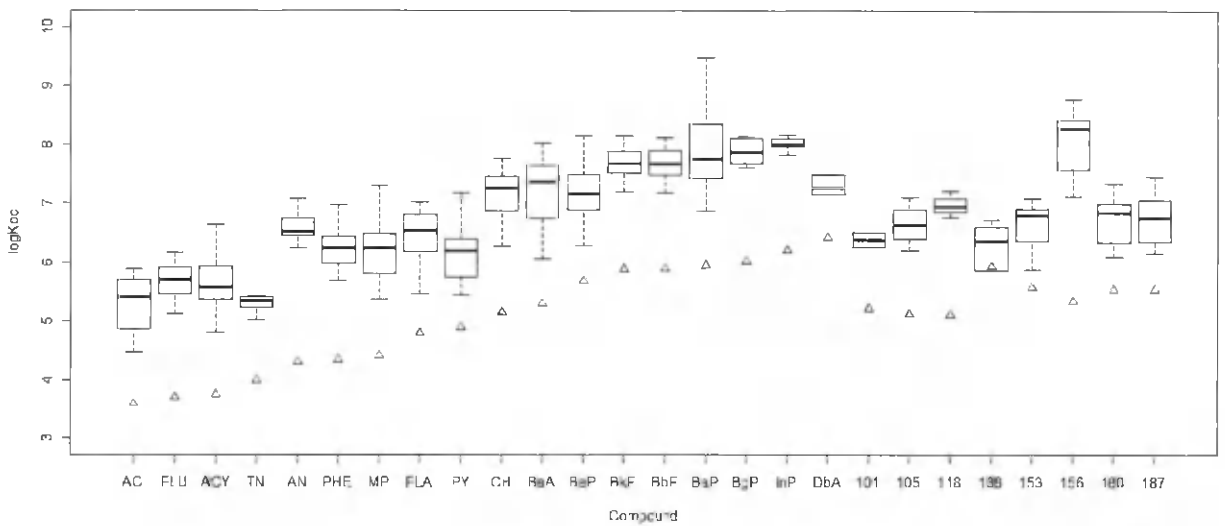

Figure C.5: Log K_{oc} values of the studied compounds using sediment concentration data. Triangles represent literature log K_{oc} values, boxplots represent the range of field-based log K_{oc} values (i.e. log $K_{oc,field}$) determined in this study using ***sediment*** *concentration data. AC: acenaphthene, FLU: fluorene, ACY: acenaphthylene, TN: 2,3,5-trimethylnaphthalene, AN: anthracene, PHE: phenanthrene, MP: 1-methylphenanthrene, FLA: fluoranthene, PY: pyrene, CH: chrysene, BaA: benzo(a)anthracene, BeP: benzo(e)pyrene, BbF: benzo(b)fluoranthene, BaP: benzo(a)pyrene, BgP: benzo(ghi)perylene, InP: indeno(1,2,3-cd)pyrene, DbA: dibenz(a,h)anthracene; PCB congeners are represented by their respective congener number.*

*Figure C.6: Log K_{OC} values of the studied compounds. Triangles represent literature log K_{OC} values, boxplots represent the range of field-based log K_{OC} values (i.e. log $K_{OC,field}$) determined in this study using **SPM** concentration data. AC: acenaphthene, FLU: fluorene, ACY: acenaphthylene, TN: 2,3,5-trimethylnaphthalene, AN: anthracene, PHE: phenanthrene, MP: 1-methylphenanthrene, FLA: fluoranthene, PY: pyrene, CH: chrysene, BaA: benzo(a)anthracene, BeP: benzo(e)pyrene, BbF: benzo(b)fluoranthene, BaP: benzo(a)pyrene, BgP: benzo(ghi)perylene, InP: indeno(1,2,3-cd)pyrene, DbA: dibenz(a,h)anthracene; PCB congeners are represented by their respective congener number.*

C.7 Graphics of biota equilibrium modelling results

Figure C.7 graphically presents the equilibrium modelling results for estimating PAH and PCB concentrations in biota as obtained in this study.

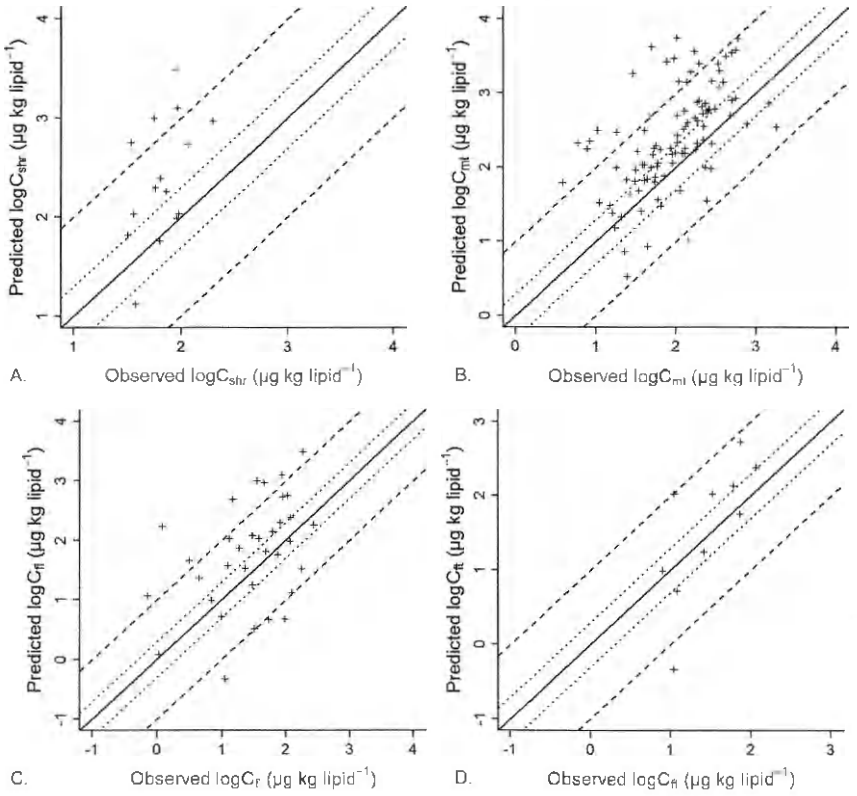

Figure C.7: Predicted vs. observed concentrations of PAHs and PCBs in biota based on logK_{ow}. The solid line represents the 1:1 relationship, dotted and dashed lines represent a deviation of a factor of 2 and 10, respectively. A. Shrimp; B. Mussels; C. Fish liver; D. Fish tissue.

D

Supporting data for Chapter 6

D.1 Passive sampling scheme

Table D.1 presents the stations that were included in the passive sampling campaigns used for Chapter 6.

Table D.1: Stations included in the passive sampling campaigns used for Chapter 6. x: station included; –: station not included.

	2007	2008		2009	
Station	Aug–Oct	May–Jul	Oct–Dec[1]	Aug–Oct	Mar–May[1]
A2	x	–	–	–	–
RV	–	x	–	x	x
NP1	x	x	–	x	–
NP2	x	x	x	x	x
OO1	x	x	–	–	–
OO2	x	x	x	x	x
OO3	x	x	–	x	–
ZB2	x	x	x	x	x
ZB3	x	x	x	x	x

[1] This sampling campaign was part of a biomarker experiment.

D.2 Calculation of the correction factor to determine log $K_{oc,field}$ from log K_{oc}

Table D.2 lists literature log $K_{oc,field}$ values of PAHs and PCBs in comparison to experimental log K_{oc} data. The difference between these to parameters is given and the average difference is reported for PAHs and PCBs separately (indicated in bold font in Table D.2).

Table D.2: Conventional (i.e. lab derived) log K_{oc} values and log $K_{oc,field}$ values determined in situ for PAHs and PCBs. The third column presents the difference between both parameters. The average difference – as determined separately for PAHs and PCBs – is given in bold italics.

Compound	log K_{oc}	log $K_{oc,field}$	log $K_{oc,field}$ - log K_{oc}
acenaphthylene	3.75	5.71	1.96
acenaphthene	3.59	4.82	1.23
fluorene	3.7	5.24	1.54
phenanthrene	4.35	5.74	1.39
anthracene	4.31	6.29	1.98
pyrene	4.9	6.2	1.3
fluoranthene	4.8	6.2	1.4
benzo(a)anthracene	5.3	6.95	1.65
chrysene	5.15	6.93	1.78
benzo(e)pyrene	5.68	7	1.32
benzo(a)pyrene	5.95	7.13	1.18
benzo(ghi)perylene	6.02	7.76	1.74
indeno(1,2,3-cd)pyrene	6.2	7.96	1.76
benzo(b)fluoranthene	5.90	7.42	1.52
benzo(k)fluoranthene	5.77	7.42	1.65
dibenz(a,h)anthtracene	6.22	7	0.78
			1.51±0.31
PCB18	4.4	5.82	1.42
PCB28	4.63	5.83	1.20
PCB31	4.68	5.87	1.19
PCB44	4.9	5.87	0.97
PCB52	4.85	5.94	1.09
PCB101	5.22	6.03	0.81
PCB105	5.12	6.07	0.95
PCB118	5.11	6.08	0.97
PCB138	5.93	6.20	0.27
PCB153	5.58	6.26	0.68
PCB156	5.33	6.28	0.95
PCB170	5.55	6.36	0.81
PCB180	5.54	6.36	0.82
PCB187	5.54	6.36	0.82
			0.93±0.27

D.3 Risk assessment data

Tables D.3 to D.6 summarise the ranges of RCRs of PAHs and PCBs for the water column, sediments, organisms and human health, respectively, as determined in Chapter 6 .

Table D.3: Ranges of RCRs of PAHs and sum of PCBs in the offshore and harbour water columns, respectively. % RCR ≥ 1 indicates the percentage of cases in which the RCR exceeded unity, indicating a risk of the compound(s) for the aquatic compartment. M: based on measured data. P: based on predicted data (based on passive sampling).

			RCR – Sea				RCR – Harbours			
Compound	PNEC (g L^{-1})	M/P	min	median	max	% RCR ≥ 1	min	median	max	% RCR ≥ 1
Acenaphthene	0.06	M	0.001	0.008	0.127	0.0	0.001	0.032	**2.598**	2.4
		P	0.004	0.005	0.013	0.0	0.002	0.069	**1.017**	3.6
Acenaphthylene	4	M	0.000	0.000	0.001	0.0	0.000	0.000	0.012	0.0
		P	0.000	0.000	0.000	0.0	0.000	0.000	0.002	0.0
Anthracene	0.1	M	0.001	0.004	0.018	0.0	0.003	0.015	0.160	0.0
		P	0.000	0.001	0.002	0.0	0.002	0.005	0.031	0.0
Benzo(a)anthracene	0.3	M	0.000	0.001	0.016	0.0	0.000	0.005	0.043	0.0
		P	0.000	0.000	0.000	0.0	0.000	0.001	0.018	0.0
Benzo(a)pyrene	0.05	M	0.001	0.012	0.210	0.0	0.001	0.068	0.480	0.0
		P	0.000	0.000	0.003	0.0	0.000	0.005	0.063	0.0
Benzo(b)fluoranthene & Benzo(k)fluoranthene	sum = 0.03	M	0.023	0.160	**1.530**	15.4	0.003	0.515	**3.067**	33.3
		P	0.000	0.004	0.027	0.0	0.001	0.027	0.192	0.0
Benzo(ghi)perylene & Indeno(1,2,3-cd)pyrene	sum = 0.002	M	0.007	0.073	0.627	0.0	0.030	0.208	**1.533**	2.4
		P	0.000	0.001	0.002	0.0	0.006	0.013	0.108	0.0
Chrysene	1	M	0.000	0.003	0.006	0.0	0.000	0.002	0.017	0.0
		P	0.000	0.000	0.000	0.0	0.000	0.000	0.006	0.0
Dibenz(a,h)anthracene	0.5	M	0.000	0.001	0.012	0.0	0.000	0.002	0.026	0.0
		P	0.000	0.000	0.000	0.0	0.000	0.001	0.004	0.0
Fluoranthene	0.1	M	0.005	0.017	0.058	0.0	0.010	0.045	0.507	0.0
		P	0.006	0.008	0.018	0.0	0.006	0.042	0.703	0.0
Fluorene	2	M	0.000	0.001	0.002	0.0	0.000	0.001	0.010	0.0
		P	0.000	0.001	0.001	0.0	0.000	0.001	0.009	0.0
Naphthalene	1.2	M	0.008	0.016	0.082	0.0	0.001	0.005	0.016	0.0
		P	–	–	–	–	–	–	–	–
Phenanthrene	0.1	M	0.007	0.017	0.054	0.0	0.005	0.030	0.177	0.0
		P	0.014	0.017	0.037	0.0	0.005	0.026	0.277	0.0
Pyrene	0.04	M	0.010	0.031	0.108	0.0	0.015	0.091	0.763	0.0
		P	0.003	0.010	0.033	0.0	0.014	0.080	0.765	0.0
Sum7PCBs	0.002	M	0.175	0.175	0.200	0.0	0.175	0.175	**1.150**	5.0
		P	0.026	0.076	0.315	0.0	0.086	0.376	**1.887**	14.3

Table D.4: Ranges of RCRs of PAHs and sum of PCBs in the offshore and harbour sediments, respectively. % RCR ≥ 1 indicates the percentage of cases in which the RCR exceeded unity, indicating a risk of the compound(s) for the sediment compartment. M: based on measured data, P: based on predicted data (based on passive sampling).

			RCR – Sea				RCR – Harbours			
	PNEC (µg kg^{-1} DW)	M/P	min	median	max		min	median	max	
Acenaphthene	0.16	M	**7.81**	**15.63**	**56.25**	100.0	**18.75**	**67.81**	**836.25**	100.0
		P	**5.87**	**6.57**	**18.45**	100.0	**3.31**	**136.20**	**1677.61**	100.0
Anthracene	50	M	0.03	0.22	0.58	0.0	0.23	0.76	**8.48**	34.1
		P	0.00	0.04	0.07	0.0	0.05	0.28	**18.10**	14.3
Benzo(a)anthracene	100 (p)	M	0.01	0.18	0.45	0.0	0.29	0.56	**22.93**	25.0
		P	0.01	0.03	0.09	0.0	0.05	0.57	**5.24**	20.8
Benzo(a)pyrene	100 (p)	M	0.01	0.25	0.64	0.0	0.32	0.67	**25.44**	25.0
		P	0.00	0.08	0.29	0.0	0.00	0.42	**18.96**	25.0
Benzo(b)fluoranthene	140	M	0.02	0.29	0.77	0.0	0.38	0.96	**24.56**	43.2
		P	0.01	0.20	0.87	0.0	0.03	0.64	**4.45**	25.0
Benzo(k)fluoranthene	140	M	0.01	0.15	0.42	0.0	0.22	0.50	**14.28**	20.5
		P	0.01	0.10	0.43	0.0	0.03	0.25	**2.18**	8.3
Benzo(ghi)perylene	84	M	0.02	0.44	0.79	0.0	0.26	0.80	**12.51**	31.8
		P	0.02	0.08	0.13	0.0	0.08	0.48	**2.85**	14.3
Indeno(1,2,3-cd)pyrene	63	M	0.02	0.64	**1.70**	29.6	0.39	**1.34**	**22.17**	84.1
		P	0.07	0.17	0.27	0.0	0.06	0.91	**5.24**	28.6
Chrysene	100 (p)	M	0.01	0.29	0.61	0.0	0.33	0.71	**22.30**	25.0
		P	0.04	0.10	0.13	0.0	0.05	0.50	**8.40**	25.0
Fluoranthene	500 (p)	M	0.01	0.08	0.32	0.0	0.10	0.41	**12.84**	20.5
		P	0.06	0.10	0.19	0.0	0.01	0.35	**6.62**	21.4
Naphthalene	50	M	0.08	0.62	**1.96**	25.9	0.56	**1.75**	**18.02**	68.2
		P	–	–	–	–	–	–	–	–
Phenanthrene	100	M	0.06	0.30	0.74	0.0	0.24	0.88	**10.35**	38.6
		P	0.29	0.36	0.78	0.0	0.10	0.66	**17.55**	17.9
Pyrene	50 (p)	M	0.03	0.51	**1.38**	14.8	0.62	**1.62**	**21.98**	90.9
		P	0.14	0.54	**1.63**	25.0	0.32	**3.04**	**34.28**	85.7
Sum 7 PCBs	1 (p)	M	0.35	**14.08**	**20.00**	44.4	**17.50**	**17.50**	**77.00**	100.0
		P	**1.28**	**6.48**	**21.63**	100.0	**2.61**	**16.39**	**78.76**	100.0

Table D.5: Ranges of RCRs of PAHs and sum of PCBs in the offshore and harbour organisms, respectively. % RCR ≥ 1 indicates the percentage of cases in which the RCR equalled or exceeded unity, indicating a risk of the compound(s) for aquatic organisms. M: based on measured data, P: based on predicted data (based on passive sampling).

	PNEC (μg kg^{-1} DW)	M/P	RCR – Sea				RCR – Harbours			
			min	median	max					
Anthracene	5	M	–	–	–	–	–	–	–	–
		P	0.001	0.011	0.019	0.000	0.015	0.049	0.311	0.0
Benzo(a)pyrene	5000	M	–	–	–	–	–	–	–	–
		P	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.0
Fluoranthene	100	M	–	–	–	–	–	–	–	–
		P	0.014	0.023	0.044	0.000	0.009	0.084	**1.563**	3.8
Naphthalene	500	M	–	–	–	–	–	–	–	–
		P	–	–	–	–	–	–	–	–
Phenanthrene	5000	M	–	–	–	–	–	–	–	–
		P	0.000	0.000	0.000	–	0.000	0.000	0.003	0.0
Pyrene	100	M	0.026	0.069	0.117	0.000	0.078	0.383	**5.824**	30.6
		P	0.003	0.012	0.035	0.000	0.015	0.066	0.738	0.0
sum7PCBs	5	M	**3.876**	**5.963**	**6.667**	100.000	**7.615**	**24.080**	**79.102**	100.0
		P	**1.220**	**5.429**	**20.203**	100.000	**2.642**	**11.510**	**82.961**	100.0

Table D.6: Ranges of RCRs of benzo(a)pyrene and sum of PCBs for secondary poisoning of humans due to seafood consumption. Data are presented for organisms sampled at both the offshore and the harbour locations. % RCR ≥ 1 indicates the percentage of cases in which the RCR exceeded unity, indicating a risk for human health. M: based on measured data, P: based on predicted data (based on passive sampling).

			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	0.439	**2.102**	**6.614**	75.0	0.854	**3.894**	**27.168**	96.4
	B	M	0.575	0.996	**1.140**	55.6	0.733	**3.864**	**12.475**	93.2
		P	**1.101**	**5.274**	**16.597**	100.0	**2.144**	**9.771**	**68.180**	100.0
	F	M	0.781	**1.312**	**3.480**	80.0	–	–	–	–
		P	**1.348**	**6.455**	**20.313**	75.0	**2.624**	**11.959**	**83.446**	100.0
Benzo(a)pyrene	S	M	–	–	–	–	–	–	–	–
		P	0.000	0.013	0.049	0.0	0.000	0.078	**1.131**	4.2
	B	M	–	–	–	–	–	–	–	–
		P	0.001	0.017	0.063	0.0	0.000	0.066	0.875	0.0
	F	M	–	–	–	–	–	–	–	–
		P	0.002	0.074	0.274	0.0	0.002	0.339	**4.920**	25.0

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Summary

Chemicals, natural as well as manmade, are a part of our everyday life. Some of these substances are vital, while others have benefits and some are detrimental to life. Nonetheless, even the vital chemicals can cause negative effects on environmental and human health if their concentrations become high enough. In Europe, approximately 70,000 chemicals are produced and/or imported and of most of them a (small) fraction ends up in the environment during production or use. In order to protect environmental and human health, it is necessary to establish at which concentrations these chemicals start causing adverse effects (the "effect assessment"), if they are present in the environment (the "exposure assessment") and if their environmental levels exceed these so-called effect thresholds (the "risk assessment"). The scientific disciplines that aim to answer these questions, are (eco)toxicology and chemistry.

The exposure assessment. In order to establish the levels at which contaminants are present in the environment, monitoring campaigns are conducted. For conventional monitoring campaigns in the marine environment, grab samples of water, sediment and biota are taken onboard oceanic research vessels. In the laboratory, these samples are then extracted and chemically analyzed. As chemical concentrations in the environment have a high temporal variability, the **levels in one grab sample are not representative of the overall pollution levels**. As such, monitoring campaigns need to be repeated frequently. However, the **operational costs of research vessels are high. Moreover, the chemical analysis of complex matrices such as sediment and biota, is labour intensive and costly as well**.

The effect assessment. In ecotoxicology, many different types of assays exist that establish the effects of chemical substances on organisms. In the most common ecotoxicological tests, individuals of a single species are exposed to a concentration range of a toxicant after which the level of effect is determined at each concentration. The data from such experiments on different species are then combined to derive the environmental level of a chemical at which no adverse effects are expected on ecosystems (i.e. a predicted no-effect concentration or PNEC). However, difficulties can arise when performing ecotoxicity studies, most of which are related to the **inability to maintain constant exposure concentrations of the chemical during the experiment**. Indeed, substance losses can occur through adsorption to test vessel walls or organic matter, bio-uptake, evaporation and/or (bio)degradation. While certain experimental setups exist to solve these problems, they are complex and costly.

The risk assessment. When both the hazard and exposure levels of a chemical have been determined, it is established whether a risk to environmental or human health exists. This is done by determining the risk characterization ratio

(RCR), which is calculated by dividing the environmental contaminant concentration by the PNEC. An RCR$\geq$1 indicates that the contaminant is present at levels that pose a risk for the environment. In conventional risk assessments, chemicals are typically assessed individually. However, **in reality organisms are exposed to multiple chemicals at once**. When combined, chemicals can cause adverse effects even when they are present below their individual effects thresholds. This is rarely assessed and the main difficulty is that the concentrations of the individual chemicals vary constantly and thus the same is true for the mixture composition. As such, **there is an endless amount of mixtures that would need to be assessed**. Moreover, significant **gaps in ecotoxicity datasets and exposure information complicate mixture risk assessments**.

Two techniques that may help to tackle the above mentioned problems are passive dosing and passive sampling. Both techniques make use of a (solid) phase with a high affinity for either hydrophobic or hydrophylic substances. Uptake and release of substances by the phase are entirely governed by passive diffusion. These two techniques and their use in ecological risk assessment is the main focus of this doctoral thesis.

In **Chapter 2**, a contribution to filling the above-mentioned data gaps was made by generating ecotoxicity data on emerging contaminants – pharmaceuticals – in a 72h algal growth inhibition assay on a key marine species: the marine diatom *Phaeodactylum tricornutum*. Subsequently, a risk assessment of these compounds as well as of their mixtures was conducted for the Belgian marine environment. Of the 7 pharmaceuticals tested, the β-blocker propranolol showed the highest toxicity towards the test species (EC50 = 0.288 mg L^{-1}) followed by the antibiotic trimethoprim (EC50 = 5.1 mg L^{-1}). The other compounds showed moderate to low acute toxicity towards *P. tricornutum*. In the Belgian marine environment the concentrations of these pharmaceuticals were well below these ecotoxicity values, indicating no risk for the test species. When taking literature ecotoxicity data for other aquatic organisms into account, a potential ecological risk from chronic exposure to propranolol was observed at 5 locations in 2 Belgian coastal harbours. Additional risks from exposure to the pharmaceutical mixtures were not observed, but mixture RCRs up to 0.50 were calculated. As such, mixture risks may emerge when more compounds are taken into account.

In order to improve our ability to assess the ecological risks of environmental contaminant mixtures, a passive dosing methodology was developed in **Chapter 3**. Both modelling exercises and experimental studies were conducted to examine the use of PDMS sheets as a source of contaminants in ecotoxicity studies. PDMS sheets were able to generate – through passive dosing – steady concentrations of

hydrophobic compounds (log $K_d > 2$) during a 72h experiment. Less hydrophobic substances (log $K_d < 2$) are likely to become significantly depleted in the sampler, causing a deviation from equilibrium conditions. As such, the passive dosing tool designed in this chapter is most suitable for compounds with log K_d values > 2.

In **Chapter 4** the passive dosing technique developed in Chapter 3 was applied in a 72h algal growth inhibition assay with *P. tricornutum*. The samplers used in this assay had previously been deployed at dedicated locations in the Belgian marine environment. As such, the environmental contaminant mixtures collected by the samplers *in situ* were recreated in the laboratory test medium through passive dosing. The exposure concentrations achieved in the test medium reflected the average concentrations of contaminants present in the field during the period of passive sampling. As such, **the mixtures created in the laboratory medium are more representative of general pollution levels than the mixtures – obtained through grab sampling – that are used in whole-sample bioassays.** As for the compounds in the higher hydrophobicity range (log $K_d > 4.1$) equilibrium had not yet been reached after the passive sampling period, the concentrations of such compounds in the test medium was lower than their average environmental concentrations. This shortcoming may be solved in the future by using a combination of different passive sampling materials.

For a majority of the mixtures, a low to moderate growth stimulation was observed in the test species. In 4 cases however, severe growth inhibition was observed (i.e. 66–100%). A mixture risk assessment, in which contaminant concentrations – obtained through conventional monitoring at the passive sampling stations – of 78 compounds from 8 different chemical groups were used, did not indicate that such severe growth inhibition could be expected in *P. tricornutum*. Potential explanations for the observed growth inhibition include (1) the presence of unknown compounds on the samplers (anthropogenic contaminants and/or marine toxins) or (2) the general lack of appropriate ecotoxicity data used for the mixture risk assessment.

Overall, the passive dosing methodology presented in this chapter provides a useful tool to expose organisms to realistic environmental contaminant mixtures which **can be of practical use in tiered mixture risk assessment approaches**.

In **Chapter 5** freely dissolved concentrations (C_{diss}) of PAHs and PCBs in the Belgian coastal zone were derived from passive sampling. C_{diss} and relevant partitioning coefficients from literature were subsequently used as the key input parameters in equilibrium models to predict the whole water concentrations (C_{ww}) of these compounds as well as their concentrations in sediment, suspended particulate matter (SPM) and biota. The modelling results were compared to analytical data obtained through conventional chemical analysis of grab samples from the

same area.

In general, contaminant concentrations were predicted well for whole water and biota. C_{ww} was increasingly underpredicted as K_{oc} increased, possibly because of the presence of black carbon. Concentrations in biota were overestimated by the equilibrium approach when log K_{ow} exceeded 6.5, suggesting an increasing role of transformation processes. Concentrations of PAHs and PCBs in sediment and SPM were consistently underpredicted although a good correlation between measured and predicted values was observed. This was potentially due to the use of experimental K_{oc} values which have been found to underestimate partitioning of hydrophobic substances to sediment in field studies.

Overall, the results showed that a passive sampling approach combined with a simple equilibrium partitioning model can function as a baseline model to predict contaminant concentrations in environmental matrices. This can in the future **offer a potential alternative for conventional monitoring approaches and may thus help reduce the associated work and costs.**

In **Chapter 6**, an environmental and human health risk assessment of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) was conducted on the basis of the modelled concentration data obtained in Chapter 5 and was subsequently compared to the results of a conventional risk assessment. In general, the risks predicted on the basis of passive sampling data were well able to cover the range of risks observed by conventional methods for all environmental compartments that were included in the assessment. Some differences were observed for the aquatic compartment and for biota. If these differences are taken into account, the proposed methodology may already be suitable as a rapid, cheap methodology to monitor risks of contaminants at sites of good chemical status.

Chapter 7 summarizes the conclusions obtained in Chapters 2 to 6 and offers suggestions for future research. In this thesis, new methodologies were developed to (1) expose test organisms in laboratory medium to environmentally relevant contaminant mixtures and (2) to estimate whole water concentrations of contaminants as well as their concentrations in sediment, SPM and biota. The latter were successfully used to perform an environmental and human health risk assessment of contaminants occurring in the Belgian marine environment. Further research could focus on the use of emerging analytical such as high-resolution full scan analysis in order to identify a broader range of contaminants in the passive sampler extracts. This may help to elucidate the adverse effects observed in the passive dosing experiment conducted in Chapter 4. For these experiments, a combination of different passive sampler materials can aid in achieving environmentally relevant concentrations for a broader range of contaminants. In order to improve the equilibrium modelling results obtained in Chapter 5, (1) the equilibrium models

can be refined (e.g. by inclusion of black carbon for the estimation of contaminant concentrations in sediment and SPM) or (2) different, more complex models may be used.

Samenvatting

Chemische stoffen, zowel natuurlijke als synthetische, maken een belangrijk deel uit van ons dagelijkse leven. Sommige van deze stoffen zijn van levensbelang, andere dragen bij aan de verhoging van onze levensstandaard en nog andere zijn schadelijk. Toch kunnen ook de eerste en tweede groep schade toebrengen aan de gezondheid van mens en milieu wanneer de blootstellingsconcentraties te hoog worden. In Europa worden naar schatting 70,000 verschillende chemische stoffen geproduceerd en/of geïmporteerd. Van de meeste van deze stoffen eindigt minstens een kleine fractie in het milieu tijdens de productie en/of het gebruik. Om mens en milieu te beschermen, is het nodig om vast te stellen aan welke concentraties deze chemische stoffen schadelijk zijn (de "effect-beoordeling"), of ze aanwezig zijn in het milieu (de "blootstellingsevaluatie") en of hun concentraties de veiligheidsnormen afgeleid in de effect-beoordeling overschrijden (de "risico-analyse"). De wetenschappelijke disciplines die zich bezighouden met deze vragen zijn de (eco)toxicologie en de scheikunde.

De blootstellingsevaluatie. Onderzoek naar de concentraties van chemische stoffen in het milieu gebeurt aan de hand van monitoring campagnes. Tijdens conventionele monitoring campagnes in het mariene milieu worden – aan boord van een onderzoeksschip – puntstalen verzameld van water, sediment en organismen. In het laboratorium worden deze stalen dan geëxtraheerd en chemisch geanalyseerd. Daar de concentraties van chemische stoffen in het milieu een grote variatie vertonen in de tijd, zijn **de chemische concentraties gemeten in één puntstaal echter niet representatief voor het algemene vervuilingsniveau**. Hierdoor dienen monitoring campagnes regelmatig herhaald te worden. De **operationele kosten van onderzoeksschepen liggen echter hoog. Bovendien is ook de chemische analyse van complexe matrices zoals sediment en organismen arbeidsintensief en kostelijk.**

De effect-beoordeling. In de ecotoxicologie worden verschillende soorten testen gebruikt om de effecten van chemische stoffen op organismen te bepalen. In de meest toegepaste ecotoxicologische testen worden individuen van eenzelfde soort voor een welbepaalde tijd blootgesteld aan een concentratiereeks van een chemische stof. Nadien worden bij elke concentratie de effecten beoordeeld. De resultaten van zulke testen uitgevoerd op verschillende organismen worden dan gecombineerd om een omgevingsconcentratie van een chemische stof te bepalen waarbij geen negatieve effecten verwacht worden voor het milieu: een milieunorm. Er treden echter vaak problemen op bij het uitvoeren van ecotoxiciteitstesten. Veelal zijn deze problemen gerelateerd aan **de moeilijkheid om de concentraties van de toxicant constant te houden tijdens het experiment**. Er kan immers een (groot) deel van de chemische stof verdwijnen via verschillende processen zoals (1) adsorptie aan de wanden van de testvat of aan organisch materiaal, (2)

(bio)degradatie, (3) evaporatie en/of (4) sterke opname in het testorganisme. Er bestaan wel specifieke testmethodes om deze verliezen tegen te gaan, maar deze zijn doorgaans complex en kostelijk.

De risico-analyse. Wanneer de milieunorm en omgevingsconcentratie van een chemische stof zijn vastgesteld, wordt er bepaald of er een risico bestaat voor de gezondheid van mens of milieu. Dit risico wordt doorgaans berekend door de omgevingsconcentratie te delen door de milieunorm. Deze verhouding wordt in het Engels de *risk characterisation ratio* (RCR) genoemd. Wanneer de RCR$\geq$1, is dit een indicatie dat er een mogelijk risico bestaat voor het milieu. In conventionele risico-analyses worden chemische stoffen doorgaans afzonderlijk beoordeeld. **In de realiteit worden organismen echter gelijktijdig blootgesteld aan meerdere stoffen**. De chemische stoffen in zulke mengsels kunnen samen schadelijke effecten veroorzaken, ook wanneer de concentraties van de individuele stoffen lager zijn dan hun respectievelijke milieunormen. Deze mengseleffecten worden maar zelden beoordeeld. De grootste moeilijkheid is de voortdurende variatie van de milieconcentraties van chemische stoffen. Hierdoor dient in theorie een nagenoeg oneindig aantal mengsels geëvalueerd te worden. Bovendien zijn er aanzienlijke hiaten in de gegevens over zowel ecotoxiciteit als blootstelling, wat de risico-beoordeling van chemische mengsels verder bemoeilijkt.

Twee technieken die kunnen helpen om bovenvermelde problemen aan te pakken, zijn passieve dosering en passieve bemonstering. Beide technieken maken gebruik van een (vast) materiaal dat een hoge affiniteit heeft voor hydrofobe of hydrofiele stoffen. Opname in en afgifte van stoffen uit deze materialen gebeurt door passieve diffusie. Het gebruik van beide technieken in ecologische risicoevaluaties staat centraal in dit onderzoek.

In **Hoofdstuk 2** werden ecotoxiciteitsdata gegenereerd voor farmaceutische stoffen aan de hand van een 72 uur groei-inhibitie test met het mariene kiezelwier *Phaeodactylum tricornutum*. Farmaceutische stoffen zijn opkomende contaminanten waarvoor de kennis rond de effecten op het milieu momenteel onvoldoende is. Zodoende draagt dit hoofdstuk bij tot het opvullen van bovenvermelde hiaten in ecotoxiciteitsgegevens. Vervolgens werd een risico-evaluatie uitgevoerd voor deze stoffen en hun mengsels voor het Belgische mariene milieu. Van de 7 farmaceutische stoffen die getest werden, vertoonde propranolol de hoogste toxiciteit voor het testorganisme (EC50 = 0.288 mg L^{-1}) gevolgd door trimethoprim (EC50 = 5.1 mg L^{-1}). De 5 andere vertoonden een matige tot lage toxiciteit voor *P. tricornutum*. In het Belgische mariene milieu bleven de milieuconcentraties van deze stoffen ver beneden de bepaalde ecotoxiciteitswaarden, waardoor geen risico voor het testorganisme aanwezig was. Op basis van additionele ecotoxiciteitsgegevens

uit de literatuur werden milieunormen berekend voor de 7 farmaceutische stoffen. Deze normen toonden een mogelijk risico voor mariene ecosystemen door chronische blootstelling aan propranolol. Dit werd waargenomen op 5 staalnamelocaties verspreid in 2 Belgische kusthavens. Er werden geen additionele risico's waargenomen door blootstelling aan de mengsels van de farmaceutische stoffen. Toch liepen de RCR-waarden voor de mengsels op tot 0.50. Zodoende kunnen milieurisico's uitgaande van chemische mengsels mogelijk toch geïdentificeerd worden wanneer meer chemische stoffen in rekening gebracht zouden worden.

Om ons vermogen tot inschatting van de ecologische risico's uitgaande van chemische mengsels te verhogen, werd in **Hoofdstuk 3** een passieve doseringstechniek ontwikkeld. Zowel mathematische modellen als experimentele studies werden uitgevoerd om het mogelijke gebruik van PDMS als een bron contaminanten in ecotoxiciteitstesten te onderzoeken. Aan de hand van vellen van PDMS konden – via passieve dosering – stabiele concentraties van hydrofobe contaminanten (log $K_d > 2$) gegenereerd worden in een 72 uur durend experiment. De voorraad van minder hydrofobe stoffen (log $K_d < 2$) op het PDMS raakt te ver uitgeput, zodat een afwijking van de evenwichtscondities ontstaat. Zodoende is de passieve doseringstechniek ontwikkeld in dit hoofdstuk het meest geschikt voor stoffen met log K_d waarden hoger dan 2.

In **Hoofdstuk 4** werd de passieve doseringstechniek (ontwikkeld in Hoofdstuk 3) toegepast in een 72 uur groei-inhibitie test met *P. tricornutum*. De PDMS vellen die in deze test gebruikt werden, werden voordien voor passieve bemonstering uitgehangen op verschillende locaties in de Belgische kustzone. Zodoende werden de mengsels van chemische stoffen die via passieve staalname bemonsterd werden in het milieu, via passieve dosering overgebracht in het testmedium. De blootstellingsconcentraties die zodoende verkregen werden, reflecteerden de gemiddelde concentraties van de contaminanten die op de staalnameplaatsen aanwezig waren tijdens de passieve bemonstering. **Zodoende zijn de chemische mengsels die op deze wijze verkregen worden in het testmedium, meer representatief voor de algemene vervuiling dan de mengsels aanwezig in puntstalen**. Voor de meest hydrofobe stoffen (log $K_d > 4.1$) werd echter nog geen evenwicht bereikt na de passieve bemonsteringsperiode. Voor deze stoffen was de concentratie die via passieve dosering bereikt werd in het testmedium dan ook lager dan de milieuconcentraties. Deze huidige tekortkoming van de techniek kan in de toekomst opgevangen worden door het gebruik van verschillende types passieve bemonsteringsmaterialen.

Het merendeel van de mengsels veroorzaakte een lichte tot middelmatige groeistimulatie in het testorganisme. In 4 gevallen werd echter een zware groei-inhibitie vastgesteld (66–100%). Een risico-evaluatie uitgevoerd voor de mengsels waar-

bij blootstellingsgegevens – verzameld via conventionele monitoring op de passieve bemonsteringslocaties – voor 78 contaminanten uit 8 verschillende chemische groepen gebruikt werden, gaf echter aan dat deze nadelige effecten niet verwacht werden. Mogelijke verklaringen voor de geobserveerde groei-inhibitie zijn (1) de aanwezigheid van onbekende contaminanten op het PDMS of (2) een gebrek aan geschikte ecotoxiciteitsdata voor het uitvoeren van de risico-analyse.

Algemeen genomen vormt de passieve doseringstechniek voorgesteld in Hoofdstuk 4 een nuttig instrument om organismen onder gecontroleerde omstandigheden bloot te stellen aan realistische mengsels van milieucontaminanten. Zodoende kan deze methodiek van groot praktisch nut zijn voor risico-evaluaties van mengsels.

In **Hoofdstuk 5** werden de vrij opgeloste concentraties (C_{diss}) van polycyclische aromatische koolwaterstoffen (PAK's) en polychloorbifenylen (PCB's) in de Belgische kustzone bepaald via passieve bemonstering. C_{diss} werd vervolgens – samen met relevante partitie coëfficiënten – gebruikt als input in evenwichtsmodellen. Aan de hand van deze modellen werden vervolgens de totale waterconcentraties (C_{ww}) van deze stoffen berekend, alsook hun concentratie in sediment, zwevende deeltjes en organismen. De resultaten van deze berekeningen werden vergeleken met de analytische data verkregen via conventionele chemische analyse van puntstalen bemonsterd in dezelfde omgeving.

Algemeen werden de totale waterconcentraties van PAK's en PCB's en hun concentraties in organismen goed benaderd. C_{ww} werd evenwel in toenemende mate onderschat met een toenemende waarde voor K_{oc}, mogelijk veroorzaakt door de aanwezigheid van zwarte koolstof. De concentraties in organismen werden overschat voor stoffen met een log $K_{ow} > 6.5$, wat een toenemende rol van transformatieprocessen suggereert. De concentraties van PAK's en PCB's in sediment en zwevende deeltjes werden consequent onderschat, maar er was een sterke correlatie tussen gemeten en voorspelde waarden. Dit was mogelijk te wijten aan het gebruik van experimentele waarden voor K_{oc} waarvoor in het verleden reeds werd vastgesteld dat het gebruik hiervan leidt tot een onderschatting van de partitionering van hydrofobe stoffen naar o.a. sediment in veldcondities.

De resultaten behaald in Hoofdstuk 5 tonen algemeen aan dat passieve bemonsteringsdata gecombineerd met evenwichtsmodellering kan gebruikt worden als een basismodel om de concentraties van contaminanten in verschillende aquatische compartimenten te voorspellen. In de toekomst kan dit **een bruikbaar alternatief vormen voor conventionele monitoring en kan zodoende helpen om de kosten gerelateerd aan de blootstellingsevaluatie te reduceren**.

In **Hoofdstuk 6** tenslotte, werd een risico-evaluatie van PAK's en PCB's uitgevoerd voor mens en milieu op basis van de gemodelleerde concentraties uit Hoofdstuk 5 en vergeleken met de resultaten van een conventionele risico-analyse. De

risico's geobserveerd op basis van de passieve bemonsteringsdata toonden algemeen een goede weergave van de resultaten van de conventionele analyse. Dit was het geval voor alle compartimenten (water, sediment en organismen). In sommige gevallen werden verschillen waargenomen voor water en biota. Wanneer met deze verschillen rekening gehouden wordt, kan de voorgestelde methodiek mogelijk reeds gebruikt worden als een snelle en goedkope techniek om de risico's van contaminanten te controleren op plaatsen waar de vervuiling binnen aanvaardbare limieten ligt.

Hoofdstuk 7 vat de belangrijkste conclusies van deze thesis samen en geeft suggesties voor toekomstig onderzoek. In dit doctoraatswerk werden nieuwe methodes ontwikkeld op basis van **passieve dosering en bemonstering** om (1) organismen bloot te stellen aan milieurelevante mengsels van contaminanten en (2) om de concentraties van contaminanten in water, sediment en organismen op een relatief eenvoudige manier in te schatten. Die laatste werden succesvol gebruikt in een risico-evaluatie van contaminanten die voorkomen in het Belgische mariene milieu. Toekomstig onderzoek zou zich kunnen richten op het gebruik van innoverende analytische technieken die een grote groep aan contaminanten gelijktijdig kunnen analyseren en die toelaten om onbekende stoffen te identificeren. Dit kan helpen om de schadelijke effecten waargenomen in Hoofdstuk 4 te verklaren. Voor de experimenten uitgevoerd in Hoofdstuk 4 kan het gebruik van een combinatie van passieve bemonsteringsmaterialen helpen om milieurelevante concentraties voor een bredere waaier van chemische stoffen te regenereren in ecotoxicologisch test medium. Om tenslotte de modelleringsresultaten uit Hoofdstuk 5 te verbeteren, kunnen (1) de evenwichtsmodellen verfijnd worden (bvb. door rekening te houden met zwarte koolstof in sediment en zwevende deeltjes) of (2) andere, meer complexe modellen gebruikt worden.

Curriculum vitae

Personalia

Name	Michiel Claessens
Date of birth	16th of August 1982
Place of birth	Mortsel, Belgium
Mobile phone	0032 (0)497 83 45 78
E-mail	michielclaessens@gmail.com

Education

2000	Secondary school degree in Science–Mathematics Koninklijk Atheneum, Lier, Belgium
2005	Biology (MSc) University of Antwerp, Belgium
2006	Master in Environmental Sanitation Ghent University, Belgium
2007 – present	PhD student in Applied Biological Sciences Ghent University, Belgium

Professional employment

2007 – 2011	Scientific researcher at the Laboratory of Environmental Toxicology and Aquatic Ecology Ghent University, Belgium
2011 – present	Regulatory Ecotoxicologist at DuPont de Nemours Mechelen, Belgium

Publications

Peer-reviewed articles (A1)

Claessens M., De Meester S., Van Landuyt L., De Clerck K., Janssen C.R. (2011) Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin* 62(10): 2199–2204.

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A4 articles (others)

Claessens M., Rappé K., Roose P., Janssen C.R. (2010) Hoe vervuild is onze Noordzee nu eigenlijk? VLIZ – De Grote Rede 27: 3–11.

Oral presentations (presenting author)

Claessens M., Monteyne E., Roose P., Janssen C.R. (2009) The use of passive samplers as a dosing device to assess the toxicity of realistic environmental mixtures. 19th SETAC EUROPE congres, Goteborg, Sweden, May-June 2009.

Claessens M., Janssen C.R. (2009) Microplastics in our seas: an invisible danger? VLIZ Young Scientists Day, Thermae Palace, Oostende, Belgium, November 2009.

Claessens M., Janssen C.R. (2010) Plastics and marine litter. EuPC general assembly 2010, Zurich, Switzerland, May 2010.

Claessens M., De Meester S., Janssen C.R. (2010) Occurrence of microplastics in the Belgian Coastal Zone. 20th SETAC EUROPE congres, Seville, Spain, May 2010.

Monteyne E., **Claessens M.**, Roose P., Janssen C.R. (2010) Use of Passive Sampling in integrated environmental monitoring. 20th SETAC EUROPE congres, Seville, Spain, May 2010.

Poster presentations

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Van Cauwenberghe L., **Claessens M.**, Vandegehuchte M.B., Janssen C.R. (2012) Plastic waste in the Belgian coastal waters and marine invertebrate wildlife. International Conference Littoral 2012: Coasts of Tomorrow. Kursaal, Oostende, 27-29 November 2012.

Conferences and workshops

SETAC Europe 17th Annual Meeting, Porto, Portugal, May 2007.

SETAC Europe 18th Annual Meeting, Warsaw, Poland, May 2008.

SETAC Europe 19th Annual Meeting, Goteborg, Sweden, May-June 2009.

SETAC Europe 20th Annual Meeting, Seville, Spain, May 2010.

SETAC Europe 21th Annual Meeting, Milano, Italy, May 2011.

SETAC Europe 22nd Annual Meeting, Berlin, Germany, May 2012.

SETAC Europe 23rd Annual Meeting, Glasgow, Scotland, May 2013.

Scientific awards

VLIZ poster award 2008, price of the professional jury, for the poster:
Claessens Michiel, Els Monteyne, Patrick Roose and Colin R. Janssen - The use of integrative passive samplers as a source of contaminant mixtures in ecotoxicological laboratory experiments.

VLIZ oral presentation award 2009, price of the professional jury, for the presentation:
Claessens M., Janssen C.R. (2009) Microplastics in our seas: an invisible danger? VLIZ Young Scientists Day. Thermae Palace, Oostende, Belgium, November 2009.

VLIZ oral presentation award 2009, price of the public, for the presentation: **Claessens M.**, Janssen C.R. (2009) Microplastics in our seas: an invisible danger? VLIZ Young Scientists Day, Thermae Palace, Oostende, Belgium, November 2009.

Membership of professional communities

Member of the Society of Environmental Toxicology and Chemistry (SETAC) since 2007.

ISBN 978-9-0598962-1-5